INCREASING THE STABILITY AND EFFICIENCY OF BIO-SOLAR CELL ELECTRODES USING REACTIVE OXYGEN SPECIES SCAVENGERS AND REDOX POLYMER IMMOBILIZATION

by

Megan E. Stephanz

A thesis submitted to the faculty of the
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

Department of Materials Science and Engineering

The University of Utah

May 2017

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Megan E. Stephanz**

has been approved by the following supervisory committee members:

Shelley Minteer	, Chair	**02/28/2017** Date Approved
Dmitry Bedrov	, Member	**02/28/2017** Date Approved
Jeffrey Bates	, Member	**02/28/2017** Date Approved

and by **Feng Liu**, Chair/Dean of the Department/College/School of **Materials Science and Engineering**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Global energy requirements are increasing with time. While fossil fuels can be relied upon for several more centuries, they would produce vast amounts of carbon dioxide. This undesirable fact makes renewable options like bio-solar cells, which are clean, inexpensive, and take advantage of abundant solar energy, a tempting prospect. However, bio-solar cells often have very short lifetimes due to reactive oxygen products that build up during photosynthesis, as well as low efficiencies when compared with inorganic solar cell options. Past research using the enzyme reactive oxygen scavenger catalase to reduce the quantity of oxygen byproducts has had advantageous effects on bio-solar cell lifetimes, leading to interest in other types of reactive oxygen scavengers. Additionally, studies into using redox polymer matrices as immobilization or mediation for biocatalysts have reportedly increased lifetime and efficiency of bio-electrodes, respectively. For both thylakoid biocatalyst bio-anodes and photosystem I biocatalyst bio-cathodes, two reactive oxygen scavengers, ascorbic acid and activated carbon, were tested to compare their abilities at extending solar cell lifetimes with those of catalase, and two redox polymers, naphthoquinone and dimethyl ferrocene modified linear polyethyleneimines (NQ-LPEI and Fc-LPEI, respectively), were investigated to determine their abilities at both stabilizing biocatalysts through immobilization and improving electron transfer efficiency through mediation. Amperometric testing was

used to reveal which combination of biocatalyst, reactive oxygen scavenger, and redox polymer created the most advantageous increases in the lifetimes and electron transfer efficiencies of bio-solar cell electrodes. While photocurrents produced were lower than for systems using expensive and toxic osmium redox polymers, it was shown that unmodified thylakoid bio-anodes made using 1 mM of ascorbic acid could last up to 148% of the lifetime of plain thylakoid electrodes and produce a photocurrent 1103% of these unmodified thylakoid electrodes when immobilized by NQ-LPEI and supplemented with 1 mM ascorbic acid. Photosystem I bio-cathodes modified with Fc-LPEI and supplemented with catalase control enzyme maintained the highest current and lifetime found for any combination of electrode, with a lifetime 264% and a photocurrent 1375% of the values for the original, comparison standard of the unmodified, blank thylakoid electrodes.

TABLE OF CONTENTS

LIST OF FIGURES

Figures

LIST OF TABLES

Tables

ACKNOWLEDGMENTS

I would like to thank Dr. Shelley Minteer and her entire research group for having me. Not only are they a very fun group of people to work with, but they were also very helpful and generous with their time, particularly Dr. Kamrul Hasan.

I would also like to thank my boyfriend, Denver Hoggatt, who keeps me from taking myself too seriously and is always there when things get rough. Without him I would have gone mad long ago.

CHAPTER 1

INTRODUCTION

Global energy demand is expected to grow at a prodigious rate over the next century.[1] While fossil fuels can supply this demand for some time, they would do so at the cost of the planet's air quality and atmospheric carbon dioxide levels. Solar energy is one possible solution to meet global energy demands without increasing air pollution and carbon dioxide concentrations, but the most efficient semiconductor-based solar cells are often expensive and sometimes toxic. Bio-solar cells may offer a solution to these problems. Made using at least one electrode coated with a biological catalyst and connected to a complimentary cathode or anode, these photovoltaic devices use the electrons generated by photosynthesis to produce electricity. The bio-electrodes may be created by applying a concentrated solution of photosynthetic plant components, such as thylakoids (the photosynthetic membranes within the chloroplasts of plants) or photosystems (the protein complexes within the thylakoids which enable photosynthesis) to a carbon paper electrode, then immobilizing the biocatalysts onto the surface by vapor depositing a thin coating of silica or applying some other form of immobilization.

All of the materials involved with creating these bio-solar cell electrodes are cheap (coming simply from plants or readily available silica), nontoxic, and recyclable, giving them advantages over conventional semiconductor solar cell materials. However,

several major problems are limiting their widespread use. First, without a level of longevity, bio-solar cells will not be a viable energy solution. Additionally, their efficiencies cannot compete with those of current inorganic photovoltaics.

The largest problem limiting the lifetime of bio-solar cells is the instability created by the products of photosynthesis. The photosystems within thylakoids use sunlight to turn carbon dioxide and water into oxygen and carbohydrates, in the process creating the electron transport used by the solar cells to create a current. However, isolated thylakoids and photosystems have no method of removing the reactive oxygen byproducts formed by photosynthesis, and, as a result, an accumulation of reactive oxygen species slows the photosynthetic reaction and damages the components of the biocatalysts. In terms of efficiency, while photosynthetic pathways have some of the highest quantum efficiencies known to science,[2] effectively transferring electrons from these pathways to an electrode surface for human use in the form of electricity is difficult and rarely achieved.

The goals of this thesis were to design methods of reducing the harmful effects of reactive oxygen species accumulation on the lifetimes of bio-solar cell electrodes, as well as to increase their electron transfer efficiency, making them better at translating light energy into electricity. Thylakoid biocatalysts were used to make bio-solar cell anodes and photosystem I biocatalysts were used to create bio-solar cell cathodes. These two photosynthetic biocatalyst systems were tested to determine their relative advantages and disadvantages with regards to these goals. Incorporation of reactive oxygen scavenger species into the bio-electrodes was also investigated, where material scavengers are chemical substances or materials added in order to remove

unwanted reaction products. Finally, redox polymers as biocatalyst immobilization materials were tested to determine their effects on both electrode efficiency and stability in comparison to standard, unmodified silica immobilization techniques. The challenge, in this case, was to find reactive oxygen scavengers and redox polymers that could mix homogenously with the thylakoid membranes and photosystem complexes and effectively improve device stability and efficiency without interfering with electron transport or device structure.

CHAPTER 2

LITERATURE REVIEW

The Potential of Solar Energy

By midcentury, global energy consumption is expected to double from beginning century levels due to population and economic growth.[1] While the currently available fossil fuel resources (especially coal reserves) could reasonably supply this demand for several centuries, consumption of fossil fuels at the rates required to do so would drastically increase the level of carbon dioxide in the Earth's atmosphere.[1] This is undesirable from an environmental standpoint, as carbon dioxide is a greenhouse gas, meaning it can add to climate change by trapping heat within the atmosphere, and, in addition, can make the oceans more acidic, threatening marine ecosystems. Thus, the development of renewable energy sources is becoming increasingly important. Particular interest is devoted to solar energy due to its great abundance: the amount of solar energy that strikes the Earth's surface in one hour is approximately equal to the global energy consumed in one year.[2]

A number of photovoltaic devices have already been developed to use this abundant source of energy. Many of these photovoltaics utilize inorganic semiconductor materials. These devices, although they currently have the highest efficiency at

converting solar energy to electricity (10-44%), often use expensive, and frequently toxic, materials.[2] As it is highly unlikely that solar energy will become a popular substitute for fossil fuels if it continues to be much more costly, organic and biological photovoltaics have also been considered. Unlike many semiconductor technologies, organic and biological photovoltaics are nontoxic, disposable, and inexpensive.[2] Organic photovoltaics often involve conductive organic polymers or small organic molecules to produce electricity from sunlight, but have low efficiencies coming from poor charge carrier mobility and transport. In addition to being nontoxic, disposable, and inexpensive, biological photovoltaics, a less developed field of study, may solve this problem by taking advantage of photosynthesis, nature's highly evolved and efficient method for solar energy conversion.[2]

During photosynthesis, plants and certain types of bacteria use light from the sun to turn carbon dioxide and water into oxygen and carbohydrates, causing a cascade of electrons that provide an electric current in the process.[2] As seen in Figure 2-1, photosynthetic reactions are photon activated, electrochemical reduction and oxidation reactions. Within the chloroplasts or photosynthetic organelles of plants, the thylakoid membranes act as an anode and oxidize water to form energy carrying molecules like ATP and NADPH. These energy-carrying molecules then allow the Calvin Cycle, or cathode of this model, to reduce carbon dioxide into a number of carbohydrates that the plant then uses as fuel. Within the thylakoids are systems of proteins called photosystems I and II, which are the components that actually capture the energy from sunlight and convey the subsequent cascade of electrons. The quantum yields of photosystem I (PSI) and photosystem II (PSII) are close to 1 and 0.8, respectively, which

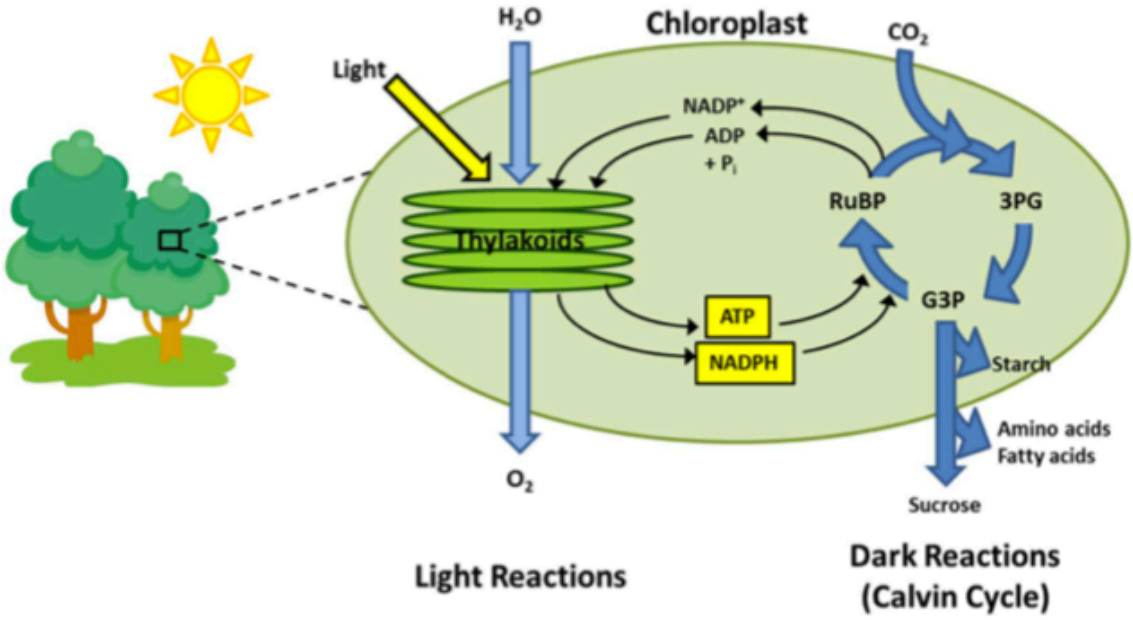

Figure 2-1. Photosynthesis modeled as a thylakoid anode with a Calvin Cycle cathode. Reproduced by permission of The Electrochemical Society and Michelle Rasmussen[2]. Copyright 2014.

is much higher than the less than 50% efficiency obtained by some of the more successful semiconductor solar cells.[2]

In order to be of use, however, there must be electron transfer between the biological catalyst and the electrode surface to which it is applied. Direct electron transfer (DET) is the ideal electron transfer method, as it moves electrons directly from the biocatalyst to the electrode without voltage loss or added instability. However, the active photosynthetic sites must be less than 1 nm away from the electrode surface to enable electron tunneling. If DET is not possible, or kinetically slow, a mediator chemical species must be added to the biocatalyst to carry electrons from the active sites to the electrode. This mediated electron transfer (MET) is often accompanied by voltage losses and instability, in addition to a reduction in environmental safety (as many mediators are toxic) and an increase in cost and complexity of the total electrode system.[2,3] The most effective mediators have redox potentials only slightly lower than the original electron source, as a lower redox potential is required to accept electrons from the source at all, but it is desirable to minimize the voltage drop that accompanies using the mediator.[3,4] Mediator species can come in a great variety, whether they are soluble species dissolved in the electrolyte or connected directly to an electrode as part of a biocatalyst immobilization system, but regardless of form, they must be able to interact with lipid layers, gain electrons from photosynthetic redox centers, and deliver those electrons to the electrode surface in order to successfully mediate biological electron sources like thylakoid membranes.[3,4]

In addition to difficulty in transferring electrons, the creation of oxygen as one of the natural byproducts of photosynthesis leads to its own problems. When a high

concentration of oxygen is trapped within a biological system, as is the case of a bio-electrode, the accumulation of product will slow down the chemical reaction itself. More importantly, the reactive oxygen species that are formed will damage the biological components performing photosynthesis, thus destabilizing the entire device.[2]

While cheap, clean, and disposable, these bio-solar cells have yet to consistently achieve the 10% efficiency needed to make them serious competitors in the photovoltaic market in terms of lifetime value. Improving photocurrent production, whether through DET or MET methods, is thus crucial to future success of bio-solar cells. Stability is also an important issue that needs to be addressed, as inefficient microbe bio-solar cells have lasted only around one year and the more efficient thylakoid and photosystem bio-solar cells have lasted only 30 or 11 h, respectively, depending on the dominant type of photosystem present.[2]

Current Progress in Bio-solar Cells

In terms of past bio-solar cell research, the three most commonly investigated photobioelectrocatalysts (or biocatalysts that use photosynthesis to enhance electricity production) are intact, photosynthetic microbes, thylakoid membranes, and isolated photosystems. Complete, photosynthetic microbes have all of the biological hardware for complete photosynthesis and can replace any damaged cell components to increase stability. However, because of the many membranes between the photosystems and electrode surface, MET is an absolute necessity. Thylakoid membranes, on the other hand, contain all the necessary components for photosynthesis but have no outer membranes, so DET is possible. Having said this, it is sometimes difficult to get their

active sites close enough to the surface, and they have less stability due to their inability to repair themselves. Photosystems are even closer to the electrode surface and allow DET, although they are unstable for similar reasons and more difficult to isolate than thylakoids.[2] Of the two separate photosystems, PSI is more desirable for device application than PSII. While both utilize solar energy to rapidly oxidize specially ligated chlorophyll a molecules, resulting in charge separations that create very large redox potentials, PSI has a quantum efficiency near unity and is considered one of the fastest and most efficient photovoltaic cells with regard to rapid photo-induced charge transfer capabilities.[5,6] While PSII's quantum efficiency of 0.8 is also impressive, DET for this photosystem is more problematic due to its active sites being located deep within the outer layer of protein, and its life expectancy is even more limited than PSI's outside of their native thylakoid membrane.[4] Additionally, of the two photosystems, PSI creates less reactive oxygen species,[5] suggesting it may have less stability problems in an isolated state than PSII. Given that thylakoids and PSI are the most stable photosynthetic options that allow potential DET, along with PSI's minimal reactive oxygen species production, these two biocatalysts were chosen for further study.

Further research determined whether biocatalysts from some sources were more efficient than others. In a study that compared the performance of bio-anodes made with thylakoids extracted from organic spinach, arugula, beets, green chard, kale, collard greens, and watercress, the spinach electrodes consistently had the highest photocurrents per chlorophyll concentration.[7] This is most likely because the soft leaves of the spinach allowed for easy cell disruption to acquire the thylakoids without much exposure to cell vacuolar sap. This is important because fatty acids inside this sap could activate

polyphenol oxidases, which would reduce photosynthetic activity. In addition to this, spinach also had a lower concentration of phenols and other inhibiting compounds than the other plants tested. This suggests that the best biocatalysts for bio-solar cell applications come from sources with limited exposure to vacuolar sap and low concentrations of phenols and other inhibiting chemicals.[7]

However, even with the most advantageous source plant used to provide bio-catalysts, the lifetimes of the bio-solar cells are still reduced by the reactive oxygen species produced during photosynthesis. In one experiment, catalase, an enzyme commonly employed to reduce the damaging effects due to reactive oxygen species production, was incorporated into thylakoid bio-anodes, as shown in Figure 2-2. The test found that activity of bio-anodes without any additives was not stable and decreased to background value after less than 10 min.[8] Bio-anodes made with the catalase scavenger, however, remained constant for approximately 30 min before gradually decreasing to 50% activity after 2 h,[8] indicating the success of catalase at reducing harmful oxygen species concentrations.

Further research into methods of increasing thylakoid bio-anode efficiency found that utilizing stroma thylakoids more than grana thylakoids increases DET with the electrode,[9] as does use of carbon quantum dots.[10] As a method of increasing surface area, thylakoid membranes in chloroplasts exist in a highly folded state. The majority of these folds consist of stacked discs, called grana thylakoids, connected by small membrane layers, called stroma thylakoids, as seen in Figure 2-3.[9] The main reason stroma thylakoids are more effective at DET than grana is that their thinner nature allows more of the photosystems on their surfaces to be close to the electrode surface than the highly

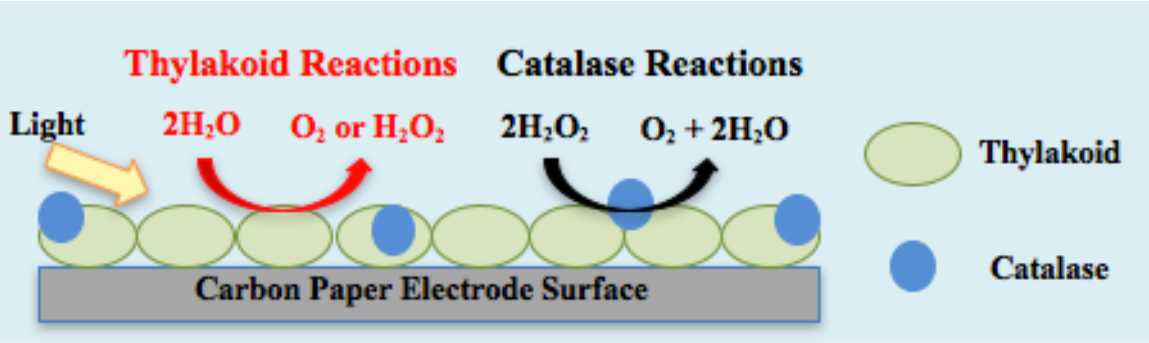

Figure 2-2. Schematic of a thylakoid bio-anode with catalase incorporated.

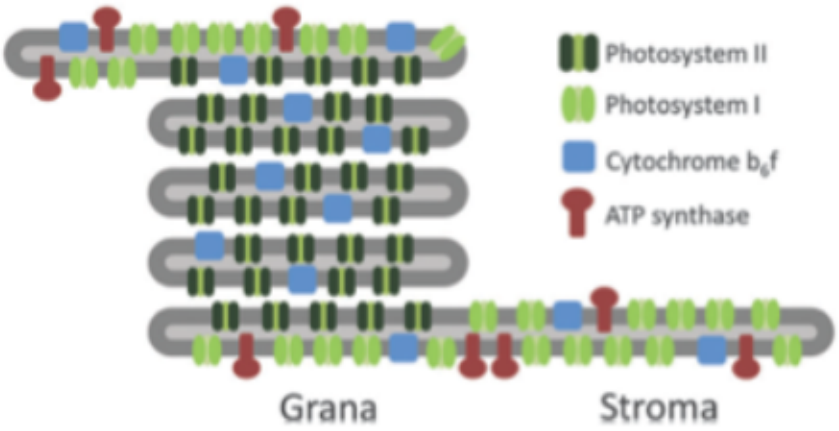

Figure 2-3. Depiction of grana vs. stroma thylakoids. Reprinted by permission of[9]. Copyright 2014, The Royal Society of Chemistry.

stacked and folded grana.[9] The stroma thylakoids also have a higher concentration of PSI per area than the grana thylakoids, which may contribute to the higher efficiency of stroma thylakoids over grana thylakoids, as well, given that PSI has a quantum yield of 1 compared to PSII's quantum yield of 0.8. In addition to increasing surface area, which allowed more DET between thylakoids and the electrode surface, carbon quantum dots can also fluoresce, allowing more of the solar spectrum encountered by electrodes to be used for solar energy conversion.[7]

Problem and Direction

The most significant hindrances to the widespread use of bio-solar cell electrodes are the short lifetimes caused by reactive oxygen species and the low efficiencies of electron transfer. This thesis focused on investigating solutions to both problems by varying biocatalysts between thylakoids and PSI, testing reactive oxygen scavengers beyond catalase, and making use of biocatalyst immobilization materials to modify electrodes. The supplemental additives and modifications used must be biocompatible with photosynthetic biology and have minimal electrochemical background.

Reactive Oxygen Species Scavengers and Their Methods

Reactive oxygen species scavengers are chemicals or materials that reduce the levels of reactive oxygen and oxygen byproducts within an environment through a variety of methods. Much of the research investigating reactive oxygen species scavengers is motivated by the food and packaging industry due to reactive oxygen species scavengers' ability to maintain food product quality by decreasing food metabolism, reducing

oxidative rancidity, inhibiting undesirable oxidation of pigments and vitamins, controlling enzymatic discoloration, and inhibiting growth of aerobic microorganisms.[11] Within this research, several varieties of scavengers have been investigated, such as metal or metal/halide systems and various nonmetallic systems including enzymatic methods, ascorbic acid, and activated carbon.[11,12]

The most commonly used metallic systems involve iron or sulfites. The majority of ferrous oxygen scavenger systems are based on the principle of iron oxidation in the presence of water. Sodium chloride and other salts are often added as catalysts to lower the humidity at which this reaction can occur.[11] Moisture permeates into iron particles, which activate and oxidize it into iron oxide, as seen in the following reactions.[12]

$$Fe \rightarrow Fe^{2+} + 2e^-$$

$$0.5\ O_2 + H_2O + 2e^- \rightarrow 2\ OH^-$$

$$Fe^{2+} + 2\ OH^- \rightarrow Fe(OH)_2$$

$$Fe(OH)_2 + 0.25\ O_2 + 0.5\ H_2O \rightarrow Fe(OH)_3$$

In terms of this method's use in a biological system, iron, while essential in low concentrations to plant growth, is highly reactive and toxic via the Fenton reaction,[13] during which iron reacts with hydrogen peroxide to form hydroxyl radicals, another reactive oxygen species.[14] Additionally, sodium is an element that can cause severe dehydration in plant biology.[15] In terms of the other metallic system mentioned, sodium sulfite reacts chemically with dissolved oxygen to form sodium sulfate.[16] However, in addition to sodium's adverse effects on plant biology, it would take a large amount of sulfite to neutralize a corresponding amount of oxygen (with a weight ratio of 8:1), the reaction works best at temperatures close to boiling, and possible byproducts include

sulfur dioxide, a toxic and odorous gas.[12,16]

Within nonmetallic reactive oxygen scavengers, there is greater variety. One reactive oxygen scavenger already in use for the purpose of improving bio-solar cell electrodes is catalase. This common enzyme is found in nearly all living organisms exposed to oxygen and catalyzes the decomposition of hydrogen peroxide to water and oxygen as seen in the following reaction.[17]

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Other enzymatic scavengers include glucose oxidase and ethanol oxidase. Glucose oxidase transfers two hydrogens from glucose to oxygen, forming gluconic acid and hydrogen peroxide.[12,18-21] Because of this peroxide product, glucose oxidase is often coupled with catalase, which then creates water and oxygen. These reactions are seen below.[18]

$$\text{glucose} + O_2 + H_2O \rightarrow \text{gluconic acid} + H_2O_2 \qquad \text{via glucose oxidase}$$

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2 \qquad \text{via catalase}$$

Oxygen scavenging systems using this glucose oxidase and catalase combination would need a constant supply of glucose and be sensitive to changes in pH and temperature, among other variables.[12] Because gluconic acid is formed as a byproduct, the solution pH of a closed system can be disturbed considerably.[18] Ethanol oxidases function similarly in that they oxidize ethanol to acetaldehyde, in the process producing hydrogen peroxide that must be neutralized by catalase.[12,22] Ethanol oxidase/catalase systems would need to be supplied with ethanol to be effective.[12] Additionally, while these enzymes effectively scavenge oxygen and thus could prevent a decreasing rate of photosynthetic reaction due to oxygen product accumulation within a system, they do

nothing without cooperation from an additional enzyme to scavenge the reactive oxygen species responsible for decreasing biocatalyst lifetimes.

Ascorbic acid, also known as vitamin C, is a nonenzymatic reactive oxygen scavenger with a method based on ascorbate oxidation to dehydroascorbic acid.[12] Food packaging systems sometimes include copper to act as a catalyst for the process, as seen in the following reactions,[12] where ascorbic acid is abbreviated as AA and dehydroascorbic acid is abbreviated as DHAA,

$$AA + 2\ Cu^{2+} \rightarrow DHAA + 2\ Cu^{2+} + 2H^{+}$$

$$2\ Cu^{+} + 2\ O_2 \rightarrow 2\ Cu^{2+} + 2\ O_2^{-}$$

$$2\ O_2^{-} + 2\ H^{+} + Cu^{2+} \rightarrow O_2 + H_2O_2 + Cu^{2+}$$

$$H_2O_2 + Cu^{2+} + AA \rightarrow Cu^{2+} + DHAA + 2\ H_2O$$

(or, summarily:) $$AA + 0.5\ O_2 \rightarrow DHAA + H_2O$$

but the reaction also naturally takes place in the biological environment.[23] Ascorbic acid provides ascorbate ions that react rapidly with superoxides, singlet oxygens, ozone, and hydrogen peroxide to neutralize these active oxygen species.[23] In addition to this, ascorbate ions help regenerate antioxidant vitamin E and carotenoid pigments used in photosynthesis.[23] Studies in plant physiology show that the monodehydroascorbate free radical oxidized from ascorbate is coupled with ATP formation in the light dependent electron transport from water (recall Figure 2-1).[24] In the presence of ascorbate, the net oxygen exchange balance is zero, while the synthesis of ATP increases two to three times due to the extra electron transport to the monodehydroascorbate molecule.[24] Additionally, studies using ascorbic acid as an oxygen scavenger in yogurt products show no perceivable change in pH while a notable decrease in oxygen and hydrogen peroxide

content.[25] Because of its oxidizing mechanism, ascorbic acid can also act as an additional electron donor to the bio-electrode system.

Activated carbon, or activated charcoal, is a more material approach to the goal of oxygen scavenging. Activated carbon is a highly porous substance that attracts and holds impurities through adsorption to its high surface area.[26] It has often been used as a cheap, cost-effective filtration method for both gaseous and liquid systems.[26,27] The powdered activated carbons are also easy to use in lab studies due to their nonreactive nature and easy suspension in liquids.[28] Although frequently used to filter out large organic molecules, not much study has gone into investigating activated carbon as a reactive oxygen scavenger. This raises some interesting questions. Activated carbon adsorption of oxygen species would not be selective, and the porosity may capture other necessary organic molecules, hindering biocatalyst function. At the same time, the high surface area provided by the carbon particles may not hinder biocatalyst function and may even increase DET with the electrode, similar to carbon quantum dot studies discussed earlier.[10]

Biocatalyst Immobilization Methods and Their Advantages

Utilizing different methods of biocatalyst immobilization onto an electrode may also increase device stability and electron transport efficiency. One of the common immobilization techniques for thylakoids and photosystems involves the use of polymer matrices. Use of poly(vinyl alcohol), or PVA, matrices was one early attempt at polymer immobilization of biomaterial, for example.[29-31] PVA bearing styrylpyridium groups was used to physically confine and entrap biological material close to the electrode surface.[29]

However, while effective at confining biological material and preventing microbial contamination of samples in a dry state, PVA's hydrophilic nature created difficulties.[29] After prolonged exposure to aqueous electrolytes, the PVA matrix would swell and break off in solution.[29,30] In addition to this, PVA's poor electrical conductivity negatively affected the biocatalysts' ability to impart electrons to the electrode surface.[30,31]

Another early attempt at using a matrix, this time of a biomaterial, to stabilize biocatalytic electrodes involved the use of a glutaraldehyde-albumin crosslinked matrix (BSA-Glu). In contrast to PVA's physical encapsulation of biological material, BSA-Glu chemically immobilized its biocatalysts. Glutaraldehyde built a network of covalent bonds with free amine groups of both biocatalyst proteins and albumin, which was itself added to prevent biocatalysts from bonding so much that they lost their biological function.[29] This biomaterial matrix was also stable in aqueous soutions.[29] The porous glutaraldehyde network acted as a diffusion barrier limiting O_2 diffusion, while at the same time retaining a large amount of electrolyte volume, allowing the introduction of experimental chemicals like inhibitors and electrons acceptors in close proximity to the biological material and permitting migration of electrons from membranes to the surface of the electrodes.[31, 32] It was found that biocatalysts immobilized by a BSA-Glu matrix had higher resistance to temperature stress, long-term storage, continuous light exposure, and changes in pH.[30,31] However, while this method of immobilization increased stability, the electron transport rate decreased from unmodified biocatalyst electrodes due to slow diffusion of membrane components entrapped by the matrix, making it undesirable from an efficiency and device standpoint.[30]

One type of immobilization method that attempts to overcome this slow electron

transfer rate is the formation of bioelectrocatalytic self-assembled monolayers, or bio-SAMs, to connect biomaterials to the electrode surface. SAMs refer to monolayers of material that attach to a substrate through some spontaneous process into an ordered structure due to lateral interactions between component molecules. These monolayers either have some electroactive property themselves or are used to organize and connect other molecules that do.[33] While bio-SAMs – whether they are composed of cystamine and pyrroloquinoline quinone, alkanethiol chains, or nickel-nitroacetic acid complexes attached to polyhistidine tags – do effectively connect biomaterial to electrodes and create much larger photocurrents than the polymer matrices discussed earlier, there is no strong evidence that the bio-SAMs have any effect on increasing the longevity of the bio-electrodes.[34-36] Additionally, many monolayers can be easily scraped or damaged and require care to use.[33]

Thus, it would be beneficial if an immobilization method could both increase the stability of the biomaterial it sequesters as well as improve, or at least not hinder, the efficiency of electron transport from the biocatalyst to the electrode surface. One such method may be the use of redox polymer matrices, where redox polymers are defined as polymers containing oxidizable or reducible functional groups covalently linked to the polymer backbone.[33] Therefore, this immobilization method could both encapsulate and stabilize the biocatalyst within a polymer matrix while also acting as a mediator to induce greater electron transport to the electrode.

One type of redox polymer that has already been used to immobilize biocatalysts are osmium (Os) redox polymers. The highly cationic nature of the Os functional groups makes these polymers strongly interact with anionic proteins and cells, forming an

electrostatic hydrogel complex that allows free diffusion of aqueous, soluble species.[37] Due to their 3-dimensional polymeric structure, the Os-cationic polymers can efficiently reach the photosynthetic reaction centers of biomaterials and allow electron transfer between neighboring redox functional groups via a hopping mechanism until the electrons reach the electrode surface.[3] Additionally, the synthetic flexibility and possibility of tuning formal redox potentials of Os polymers make them attractive for engineering better electrodes.[3,37] However, while effective, Os is rare and expensive, as well as toxic, and these qualities do not lend acceptability in trying to popularize biological photovoltaics.

Two much cheaper redox polymers which have not yet been studied in conjunction with photosynthetic catalysts are naphthoquinone and dimethyl ferrocene (which shall be referred to as ferrocene) modified linear polyethyleneimines. Polyethyleneimine polymers in general are very biocompatible due to their polyamine backbones.[38] In aqueous conditions, they exhibit a self-buffering effect where the amine groups become positively charged. The cationic environment, similar in this respect to that of Os polymers, interacts well with anionic protein sites, and the amine groups offer places to readily anchor various pendant molecules (such as naphthoquinone or ferrocene), which allows for easy modification and a cross-linking ability that enables the formation stable hydrogels for the immobilization of biocatalysts.[38] While neither naphthoquinone or ferrocene modified linear polyethyleneimines have been used to mediate photosynthetic biocatalysts, they have both been documented as having great success acting as redox mediators for electrodes dealing with enzymes like glucose oxidase and dehydrogenases, however.[39-41] Naphthoquinone-based polymer hydrogels

have been used for fuel cell applications and resulted in stable cells with continuously high potential differences,[39] as well as high current and power densities.[40] This particular redox polymer may be especially effective at mediating electrons from the PSII complexes within thylakoid biocatalysts, as the naphthoquinone functional groups can permeate through the photosynthetic membranes and may successfully mimic quinone electron acceptors located within the PSII electron transport chains.[5] Ferrocene polymers have been praised for their ease of modification, biocompatibility, stability against temperature and oxidative changes, large surface area, good electrical conductivity, and redox properties.[41,42] A ferrocene redox polymer may be especially effective at mediating electrons from PSI, as its iron-based ferrocene functional groups may successfully compete with the iron-sulfur clusters that figure prominently within PSI's own electron transport chains,[5] and its redox potential is at an advantageous level for accepting electrons from the reductive PSI biocatalyst.

Results of Literature Review

Thus, in terms of investigating various reactive oxygen scavengers beyond catalase, it was decided that ascorbic acid, based on its multiple recommendations of being beneficial to plant biology and being an effective reactive oxygen species scavenger, as well as activated carbon, an additive of unknown but possibly beneficial reactive oxygen species scavenging quality, would be tested as experimental oxygen scavengers. In terms of investigating biocatalyst immobilization methods that might increase both the longevity and efficiency of biocatalyst electrodes, naphthoquinone and ferrocene modified linear polyethyleneimine redox polymers were chosen. Redox

polymers have the advantage of providing both polymeric matrix immobilization that may protect and stabilize biocatalysts and a method of mediating electron transport to the surface of the electrode. Both naphthoquinone and ferrocene redox polymers have not yet been tested in photosynthetic systems, but are much cheaper than proven alternatives and have shown good results when applied with enzymes. They may be able to effectively mediate electrons from separate photosystem transport chains. These four experimental systems were tested with both thylakoid and PSI biocatalysts and compared against electrodes with biocatalyst alone and biocatalyst mixed with the control reactive oxygen scavenger catalase.

CHAPTER 3

INVESTIGATION OF THYLAKOID BIOCATALYST ANODES

As thylakoid photosynthetic reactions primarily involve the oxidation of water, they were used to create potential bio-solar cell anodes. Included within this chapter are all of the relevant procedures used to create, characterize, and test bio-anodes made using thylakoid biocatalysts. The sources of materials and compositions of specific buffers mentioned in the following methods can be found in the Appendix A. Following the experimental procedures, the results in terms of reactive oxygen scavenger concentration optimization and the photocurrents and lifetimes of finished experimental thylakoid bio-anodes found using amperometry are reported and discussed.

Experimental Procedures

Extraction of Thylakoids from Spinach

To begin the extraction of thylakoids, 5M chloroplast isolation buffer (CIB) as described in the Appendix was diluted to 1M concentration and allowed to chill at 40 ^{o}F. The ribs from one bunch of spinach were then removed by tearing or cutting. The torn leaves were rinsed well with ultrapure water and spun in a salad cleaner to remove excess water. Next, the cleaned spinach was put into a blender with 500 mL of diluted, chilled

CIB and blended together at maximum speed for five 1 s pulses. The resulting fluid was filtered into a beaker through three layers of cheesecloth to strain out the largest debris.

After pouring equal amounts of the strained liquid into two large centrifuge beakers, the spinach solution was centrifuged at 200g of centrifugal force for 3 min at 4 ^{o}C to remove any remaining debris. The supernatant was then centrifuged at 1000g for 7 min at 4 ^{o}C. After discarding the supernatant from this step, the pellets on the bottoms of the centrifuge beakers were resuspended in 2 mL each of the diluted CIB using a paintbrush. The suspensions were then carefully overlaid into two new centrifuge vials, each containing a mixture of 4 mL Percoll and 6 mL diluted CIB. These vials were centrifuged at 1700g for 6 min at 4 ^{o}C to obtain pellets of whole chloroplasts.

These pellets were resuspended in each vial with 20 mL of lysing solution and centrifuged at 1700g for 6 min at 4 ^{o}C for a second time to lyse the chloroplasts and expose the thylakoid membranes. Once the supernatant was carefully siphoned out of each vial, the thylakoid pellets were resuspended with 2 mL each of thylakoid resuspension buffer (TRB). Both resuspensions were then combined in one vial.

Measurement of Chlorophyll Concentrations and Storage

Immediately following thylakoid extraction, the chlorophyll concentration was determined using a ThermoFisher Scientific GENESYS™ 20 Visible spectrophotometer. Chlorophyll concentration per batch of extracted thylakoids was an important quantity, because it allowed normalization of results so that the photocurrents measured from different thylakoid batches, which may have had slightly different chlorophyll concentrations (and thus different photosynthetic ability), could be compared. It also

allowed thylakoid concentration to be estimated.

To do so, the spectrophotometer wavelength was set to a value of 652 nm, and the spectrophotometer was then zeroed at this wavelength using a cuvette filled with 990 μL of 80% acetone solution. Once the spectrophotometer had been zeroed, the vial containing the newly extracted thylakoid solution was tipped upside down several times to ensure homogenous concentrations of thylakoids throughout the vial. Following this, 10 μL of the homogenized thylakoid solution was added to the cuvette containing the acetone solution. After dispersing the thylakoids throughout the cuvette, the absorbance was measured.

The concentration of chlorophyll in mg/mL of the solution could then be found by multiplying the absorbance at 652 nm by 100 (the dilution factor) and dividing the number by 36 mL mg^{-1} (the extinction coefficient). If the overall concentration was greater than 0.2 mg/mL, TRB was used to dilute the thylakoid solution to that amount.

Once the thylakoid solution was diluted to the proper concentration, the vial was flipped upside down to ensure homogenous distribution of thylakoids throughout the volume, and 2 mL of thylakoid solution was pipetted into a 2 mL Eppendorf microcentrifuge tube. This step was repeated until all the thylakoid solution had been pipetted into microcentrifuge tubes before storing these aliquots in the freezer at 20 ^{o}F. Thylakoids stored in this manner could be unfrozen for up to a month for use in new electrodes as needed.

Preparation of Anodes Without Incorporating Redox Polymers

For blank electrodes using only unmodified thylakoid solution as a biocatalyst, a hot plate was warmed to 185 ^{o}F to begin melting wax, and a 2 mL aliquot of frozen thylakoid solution was set to thaw in a room temperature water bath. While the wax melted, a number of Toray carbon paper electrode bases were cut out with heads an area of 1 cm^2. Electrodes for all test conditions were prepared in at least triplicate to ensure enough electrodes for proper triplicate testing and extras in case of accidental breakage.

Once the wax was melted, tweezers were used to dip the stems of the carbon paper electrodes into the wax until only the heads were uncovered. The sticky wax ends were then placed on an overturned weigh boat and allowed to dry. The resulting hydrophobic, wax-coated electrode stems would ensure that any signal detected by the potentiostat in later tests could be attributed to the electrochemistry occurring at the electrode head rather than electrolyte travelling up the stem and making contact with the potentiostat connections.

While the electrodes dried, the thawed aliquot of thylakoid solution was turned over several times to ensure homogenous mixing of thylakoids and the concentration of the chlorophyll in the aliquot was measured following the procedure described earlier. If the concentration was not within 0.02 mg/mL of the desired 0.2 mg/mL mark, the thylakoid solution was diluted with the appropriate amount of TRB. After the desired chlorophyll concentration was confirmed, the aliquot was turned over several times to ensure homogenous mixture prior to pipetting 25 μL of solution onto the head of a dried electrode. The droplet of thylakoid solution was then spread evenly across the head surface. This homogenization and pipetting procedure was repeated until all electrode

heads had been covered in thylakoid solution but the plain carbon electrodes.

The weigh boat supporting the electrodes was then placed underneath a small fan and left for 1 h for the electrodes to dry. Care should be taken to keep the electrodes in shaded or dark conditions during this step to avoid premature light damage and reactive oxygen species production. Afterwards, tweezers were used to remove the electrodes from the weigh boat and place them face up in a petri dish. This petri dish was placed within the fume hood, where a cap filled with 200 μL of tetramethyl orthosilicate (TMOS) was put in the center of petri dish so that all parts of the electrodes remained uncovered. The lid of the petri dish was then lowered to allow vapor deposition of TMOS onto the electrode surfaces for 20 min. Again, care should be taken to keep the electrodes shaded during this period. The TMOS-sealed electrodes were then removed from the petri dish and stored overnight in a dark refrigerator to cure at 40 ^{o}F.

For catalase control electrodes, this procedure was exactly the same except that once the concentration of thylakoid solution had been determined to be acceptable, 0.5 mL of it were pipetted to a separate aliquot and mixed with 15 μL of catalase enzyme in solution form. This mixture was then pipetted onto the electrode heads. Catalase from *Aspergillus niger* acquired from Sigma Aldrich was used.

For the activated carbon electrodes, various suspensions were made of different concentrations of activated carbon in TRB. As no previous work incorporating activated carbon into electrodes had been done, the initial concentrations of these source solutions were 12 g/L, 60 g/L, and 120 g/L, based on sources that used activated carbon for other purposes in a range of concentrations from 12 g/L to 348 g/L.[43,44] (Note: activated carbon itself has no standard molar weight, so these concentration increments are based

on 12.01 g/mol, the molar mass of elemental carbon.) These suspensions were allowed to soak overnight for at least 12 h to let buffer displace the air in the activated carbon pores. Once the thylakoid solution concentration had been ascertained, 0.5 mL of thylakoid solution were placed into three new separate aliquots and the soaked activated carbon suspensions were vortexed to resuspend the activated carbon evenly. After this, 15 μL of each concentration was then added to its respective thylakoid aliquot, and the three mixtures were vortexed before each 25 μL were pipetted onto an electrode head to ensure a homogenous suspension of activated carbon particles. Later concentrations of activated carbon source solutions included 3 g/L, 6 g/L, and 24 g/L.

Initially, the production of ascorbic acid electrodes was approached much the same way, with initial investigation showing some studies using as little as 1mM of ascorbic acid in oxygen scavenger tests and other resources showing that levels as high as 10-25 mM of ascorbic acid were naturally present in plant chloroplasts.[45,46] Thus, similar to the procedure used to make activated carbon electrodes, three initial ascorbic acid solutions in these concentrations were made and later added in small amounts to separated quantities of thylakoid solution before deposition onto the electrode surfaces. However, results using this technique showed no increase from the lifetimes of the blank thylakoid electrodes and raised concerns over whether the acidity of the initial ascorbic acid solutions were harming the thylakoids. Eventually, it was determined that the concentration of ascorbic acid within each electrode was simply too small to have much effect on thylakoid performance. To remedy this, SEM images were taken of thylakoid electrodes to determine the volume of the dried thylakoid layer, and this volume was used to back calculate the appropriate concentrations of the starting solutions to end up with

electrodes actually reflecting the desired testing concentrations of 1mM, 10mM, and 25mM. The respective concentrations of these new source solutions were 0.034M, 0.34M, and 0.86M.

Preparation of Anodes Incorporating Redox Polymers

Thylakoid solutions were thawed, measured for chlorophyll concentration, and modified with the concentrations of experimental reactive oxygen scavengers found to be most optimal for unmodified thylakoid electrodes as described in the previous section. Similarly, Toray carbon paper electrodes were also cut out and coated up the stems with wax. Afterwards, depending on the redox polymer condition to be tested, a 10 mg/mL solution of either naphthoquinone modified linear polyethyleneimine (NQ-LPEI) or ferrocene modified linear polyethyleneimine (Fc-LPEI) was made by vortexing several mg of either polymer with the appropriate amount of water. These redox polymer solutions could be reused for several different batches of electrodes within a 24 h period. A 10% solution of ethylene glycol diglycidyl ether (EGDGE) was also made by diluting the appropriate amount of EGDGE with water. This solution acts as the cross-linker for the redox polymer being used and must be made fresh for each batch of electrodes.

Once the appropriate polymer and thylakoid test solutions were made, the redox polymer, thylakoid biocatalyst, and EGDGE fluids were mixed together with respective volumes of 21 μL to 9 μL to 1.13 μL for each test electrode being made. Each electrode head was covered with 30 μL of its respective mixture and left to dry and polymerize without use of a fan for 25 min, after which the electrodes were immediately tested.

Use of a fan was avoided during drying as it may have caused uneven distribution

of redox polymer across the electrode surfaces. Additionally, as the redox polymer itself now immobilized the biocatalysts on the surface of the electrode, no TMOS coating and curing process was necessary. Because the redox polymer matrices formed were hydrogels, and thus would swell slightly within aqueous conditions, any added inflexible coating such as TMOS could possibly have flaked off into the testing buffer during an experiment, as well.

Amperometric Testing

Anodes of various compositions were tested via amperometric testing. This means the photocurrent generated by the electrodes was recorded as a function of time. The purpose of this mode of testing was, initially, to determine which of the tested conditions for each type of reactive oxygen scavenger was most successful at increasing lifetime of thylakoid anodes, as well as to compare the lifetimes of experimental electrodes with those of the blank and control catalase conditions. The optimal reactive oxygen scavenger conditions found for unmodified thylakoid electrodes were then tested in conjunction with redox polymers and the other biocatalyst, PSI.

An open-faced aluminum box with a detachable black felt cover was placed on the counter next to a potentiostat, and a ring stand and 250 W halogen lamp were arranged inside so that the light from the lamp would shine directly into the small 20 mL beaker the ring stand held. This beaker was filled with 0.1M citrate buffer at a pH of 5.5. A platinum electrode was rinsed with DI water and placed in the beaker before being connected to the counter electrode clip of the potentiometer. A saturated Ag/AgCl reference electrode was also rinsed with DI water and used as the reference electrode in

the beaker. The working electrode clip was then connected to the first of the electrodes, making sure that the teeth of the alligator clip had pierced the wax layer on the electrode tail and were in contact with the underlying carbon. All three electrodes were arranged in the citrate buffer beaker so that none were touching and the electrode head faced directly towards the lamp under the surface of the buffer.

Once everything was situated, the lamp was turned on, and the box opening was covered with felt so that no outside light could reach the electrode. The potentiostat was also powered on. CH Instruments CHI660E test software was used to run the electrode at a potential of 0.45 V vs. the Ag/AgCl reference electrode for 300 s in the light to eliminate surface reactions on the working electrode. After the surface reaction run was complete, the electrode was run again under the same conditions except that the light alternated on and off every 100 s until a 25% reduction in photocurrent from the second, more stable photocurrent peak was observed. At this point, the test was stopped and saved before removing the test electrode and replacing with a new one. Tests were run in triplicate and time to 25% reduction of initial photocurrent, where photocurrent was determined by the difference between dark and light current conditions, was used as a measure of lifetime. The photocurrent values for all electrode conditions were normalized by the chlorophyll concentrations measured for their respective thawed biocatalyst sources, and redox polymer conditions were normalized not only in terms of chlorophyll concentration but also in terms of total biocatalyst volume to be comparable with the higher biocatalyst volumes found in the unmodified silica electrodes.

Prior to each triplicate test of experimental working electrodes, a plain carbon electrode was tested for 300 s of alternating light conditions. The amperometric results

from this electrode were then examined to determine whether any change in photocurrent occurred with changing light condition. This should not have been the case, as the plain carbon electrode had no photosynthetic biocatalysts on its surface to cause a light-dependent change. If a change was observed, this indicated an amount of error that would have to be subtracted from the photocurrents seen for actual test electrodes.

For the 25mM ascorbic acid electrodes, a light pattern of 200 s on, 200 s off was adopted to account for a slower rate of photocurrent stabilization being observed. Maintaining the pattern at the 100 s values would have resulted in unstable photocurrent peaks.

Cyclic Voltammetry

Cyclic voltammetry refers to measuring the current density of an electrode as cycles of potential are applied to it. This method of testing can reveal many different qualities of an electrochemical system, but within this research was used to show evidence of photobioelectrocatalysis occurring for new applications of biocatalyst and/or immobilization method. Thus, when investigating thylakoid bio-anodes, cyclic voltammograms (CVs) were taken of blank naphthoquinone modified linear polyethyleneimine thylakoid electrodes and blank ferrocene modified linear polyethyleneimine thylakoid electrodes.

Working, counter, and reference electrodes were arranged in the same box and light system used for the amperometric testing. Naphthoquinone modified linear polyethyleneimine working test electrodes were first held in dark conditions while the potential cycled through a range of -0.4 to 0.2 V vs. Ag/AgCl eight times at a scan rate of

0.01 V/s. Eight cycles were used to allow current patterns to stabilize from their largest values occurring during the first cycle. Afterwards, the light was turned on within the box and this procedure was repeated. Ferrocene modified linear polyethyleneimine working test electrodes were held in dark conditions and cycled through a potential range of -0.2 to 0.55 V vs. Ag/AgCl forty times at a scan rate of 0.01 V/s. A greater number of cycles were needed to reach a stable voltammogram. Shifts to lower, more negative currents with the addition of lighted conditions indicate the combination of biocatalyst and/or immobilization method increases in oxidative current when light is applied. Likewise, shifts towards more positive currents indicate a greater reductive current produced by the system in the presence of light. For the oxidizing thylakoid biocatalysts, the first pattern would be expected.

Results and Discussion

Amperometric analysis of all reactive oxygen species scavengers and redox polymers was performed. Table 3-1 summarizes the amperometric results collected. Abbreviations include AC, meaning activated carbon; AA, meaning ascorbic acid; NQ-LPEI, meaning naphthoquinone modified linear polyethyleneimine; and Fc-LPEI, meaning ferrocene modified linear polyethyleneimine. Uncertainties for the data collected are included as the standard deviation of the data measurements.

Blank and Control Anodes

Blank, unmodified thylakoid electrodes were found to last an average of 983 ± 115 s before 25% photocurrent reduction and had a relatively high normalized

Table 3-1

Amperometric results of blank, control, activated carbon, ascorbic acid, naphthoquinone redox polymer, and ferrocene redox polymer experimental thylakoid test electrodes.

Electrode Type	Lifetime in Seconds	Normalized Photocurrent in A/(mg/mL)
Blank	983 ± 115	$4.107 \pm 0.682 \times 10^{-7}$
Control (Catalase)	1083 ± 208	$2.418 \pm 0.647 \times 10^{-8}$
3 g/L AC	1117 ± 305	$3.190 \pm 0.560 \times 10^{-7}$
6 g/L AC	1117 ± 305	$2.677 \pm 0.489 \times 10^{-7}$
12 g/L AC	1383 ± 404	$2.227 \pm 0.402 \times 10^{-7}$
24 g/L AC	1450 ± 200	$1.450 \pm 0.140 \times 10^{-7}$
60 g/L AC	1117 ± 305	$1.763 \pm 0.495 \times 10^{-7}$
120 g/L AC	983 ± 503	$2.070 \pm 0.849 \times 10^{-7}$
1mM AA	1450 ± 100	$3.986 \pm 0.385 \times 10^{-7}$
10 mM AA	1183 ± 115	$4.357 \pm 0.741 \times 10^{-7}$
25 mM AA	1267 ± 306	$8.192 \pm 5.226 \times 10^{-7}$
Blank + NQ-LPEI	717 ± 115	$7.503 \pm 1.330 \times 10^{-7}$
Control + NQ-LPEI	783 ± 115	$4.845 \pm 0.817 \times 10^{-7}$
24 g/L AC + NQ-LPEI	917 ± 58	$1.120 \pm 0.028 \times 10^{-6}$
1mM AA + NQ-LPEI	950 ± 173	$1.656 \pm 0.467 \times 10^{-6}$
Blank + Fc-LPEI	433 ± 29	$7.356 \pm 2.391 \times 10^{-7}$
Control + Fc-LPEI	450 ± 29	$7.671 \pm 3.016 \times 10^{-7}$
24 g/L AC + Fc-LPEI	483 ± 58	$9.676 \pm 1.042 \times 10^{-7}$
1mM AA + Fc-LPEI	516 ± 115	$6.992 \pm 1.836 \times 10^{-7}$

photocurrent of 4.107 ± 0.682 x 10^{-7} A per mg/ml of chlorophyll. The pattern of switching the light on and off led to clear, well-defined current peaks as can be seen in Figure 3-1.

The addition of catalase from *Aspergillus niger* in the control bio-anodes seemed to extend the average lifetime of the electrodes to 1083 s, or 110% of the lifetimes of the blanks, but statistical analysis using Student T-tests at a 95% confidence level revealed this increase was not significant.[45] The average photocurrent of these electrodes, however, reduced significantly to 2.418 ± 0.647 x 10^{-8} A/(mg/mL).

Experimental Reactive Oxygen Scavenger Anodes

Results for the varying concentrations of activated carbon tested show more interesting results. Figures 3-2 and 3-3 graph the lifetimes and normalized photocurrents of these electrodes with respect to their various activated carbon concentrations. While there is no significant difference between lifetimes of the activated carbon conditions as determined through T-tests, the average lifetimes of these electrodes increase with concentration up to 24 g/L, where average lifetime is 148% that of the blank electrodes with significant difference, before decreasing with further concentration increases. The noise of the amperometry graphs also increases with concentration. This suggests that activated carbon is to some extent successful as a reactive oxygen scavenger, but at higher and higher concentrations, this scavenging advantage must be balanced with its possible light blocking tendencies. The thylakoid solutions with concentrations of activated carbon higher than 6 g/L added were noticeably darker than plain thylakoid solution. At high concentrations, the activated carbon may have been blocking the

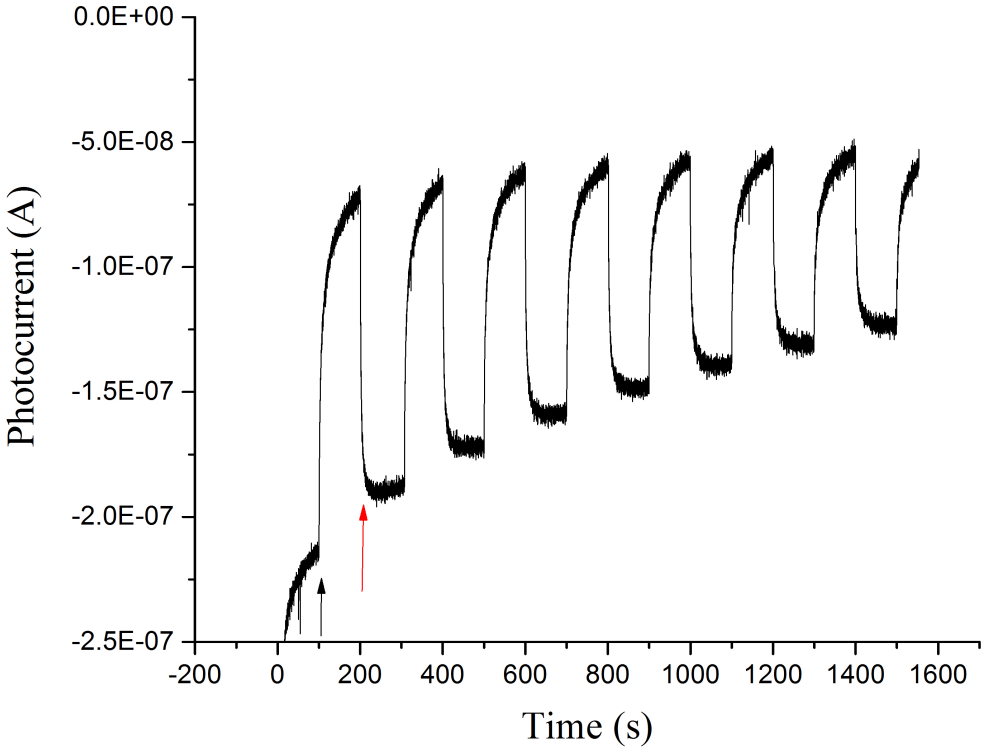

Figure 3-1. Amperometry of a blank thylakoid anode at 0.45 V vs. saturated Ag/AgCL in 5.5 pH 0.1M citrate buffer. Sharp increases in photocurrent, the first of which is marked by a black arrow, indicate turning off the lamp, and sharp decreases in photocurrent, the first of which is marked by a red arrow, indicate turning on the lamp.

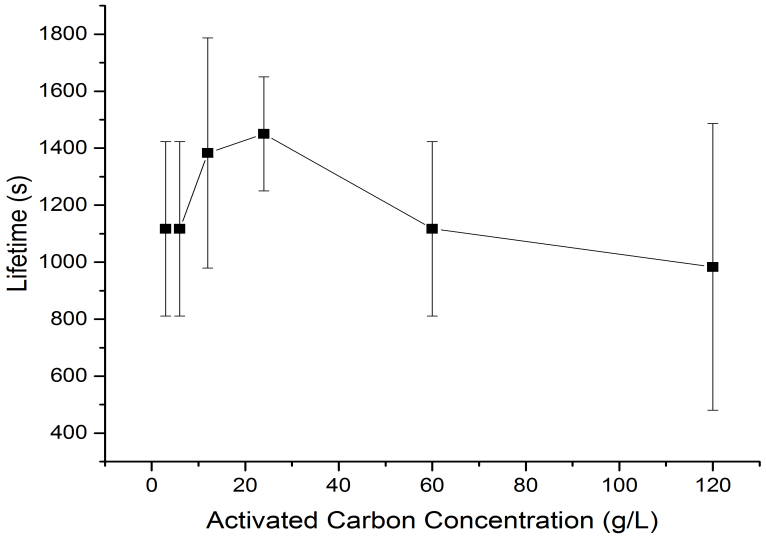

Figure 3-2. Lifetime of activated carbon electrodes show the least uncertainty and highest average around the 24 g/L activated carbon concentration, although no statistical significance is seen between lifetime values.

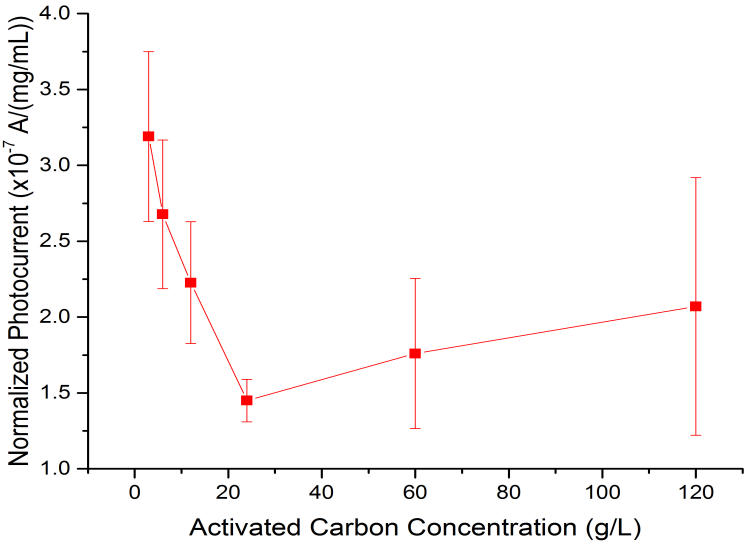

Figure 3-3. Photocurrents of activated carbon electrodes, exhibiting much lower values than blank electrode photocurrents, especially at high concentrations of activated carbon.

lamplight from reaching a large percentage of thylakoids or been providing too many competing electric pathways with that of the electrode surface, which may account for the increasing amount of noise seen in the amperometric graphs in relation to increasing concentrations. Figure 3-4 shows the relatively clean data produced by a low concentration 3 g/L activated carbon anode with the very noisy data made by a 60 g/L activated carbon anode. This increased noise makes it difficult to obtain a steady current signal from the device.

When looking at the photocurrents achieved by these electrodes, both the 24 g/L and 60 g/L conditions had significantly lower photocurrents than the highest 3 g/L photocurrent condition, and even the smallest added concentration reduces photocurrent to below that achieved by the blank electrodes, with all the greater concentrations showing photocurrents significantly lower than blank photocurrent value. This suggests that the porosity of the activated carbon is unselectively adsorbing molecules necessary to the thylakoid function along with oxygen byproducts and affecting their efficiency. The slight increase in photocurrent at 60 g/L and 120 g/L concentrations may indicate that there is a slight amount of increased DET due to surface area at these higher concentrations, but not enough to overcome the adverse effects of the carbon's porosity adsorbing biocatalyst components unselectively. Despite being lower than that of the blanks, however, all the activated carbon electrodes maintained a much higher photocurrent than those demonstrated by the catalase controls.

The 1mM ascorbic acid tests match the activated carbon's highest lifetime increase at 1450 s (148% of blank lifetime) but with higher significance. The photocurrent at this most successful ascorbic acid condition is also much less diminished

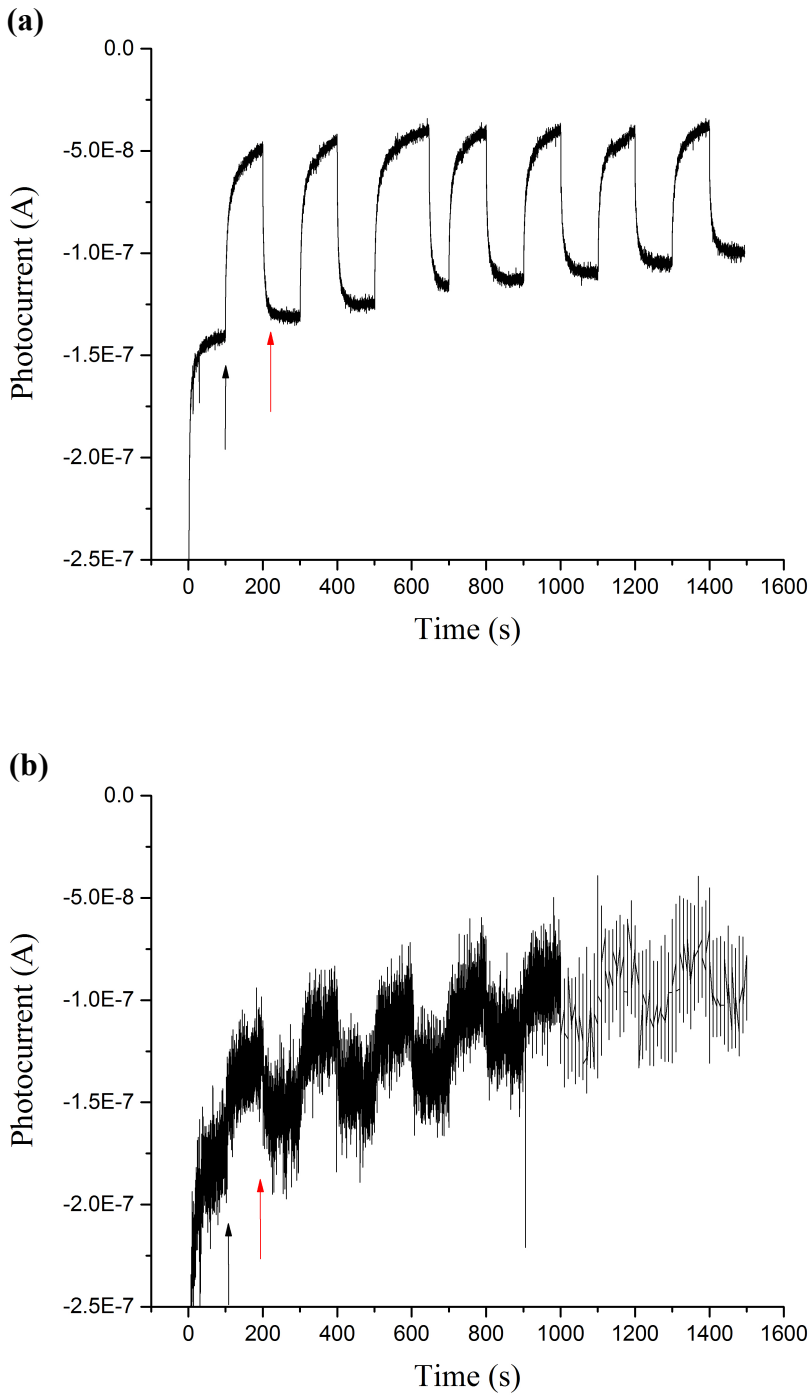

Figure 3-4. Comparison of activated carbon results obtained at 0.45 V vs. saturated Ag/AgCl in 5.5 pH 0.1M citrate buffer exhibiting the change in data quality seen with concentration. Black arrows indicate turning the light off, and red arrows indicate turning the light on. (a) Clean data from a 3 g/L activated carbon bio-anode. (b) Much noisier data gained from a 60 g/L activated carbon bio-anode.

than its activated carbon counterpart. At 3.986 ± 0.385 x 10^{-7} A/(mg/mL), there is no significant difference between this average photocurrent and the original 4.107 ± 0.682 x 10^{-7} A/(mg/mL) exhibited by the blank bio-anodes.

Increasing concentrations of ascorbic acid led to both statistically significant lowering of lifetimes and increasing of average photocurrents, although increasing standard deviations prevented any significant photocurrent change. In addition to this, the highest concentration (tested at 25mM ascorbic acid) began to show much slower photostabilization rates of its amperometry peaks, resulting in the need for 200 s lamp on and off periods to capture full photocurrent stabilization. Figure 3-5 shows a comparison of the sharp, short peaks accomplished at 1mM concentrations with the longer wait times, seen in the much greater peak lengths, required for a 25 mM electrode. All of this evidence suggests that at higher concentrations, the ascorbic acid is escaping the electrode into the citrate testing buffer (resulting in less lifetime stability), where it can act as another oxidizer in addition to the anode, thus artificially increasing photocurrent and making the photocurrent stabilization take longer due to the increased amount of oxidizing elements present.

Redox Polymer Anodes

The previous amperometry results thus revealed that the 24 g/L activated carbon bio-anodes and the 1mM ascorbic acid bio-anodes supported the longest average lifetimes for unmodified, silica immobilized thylakoids with the least uncertainty of their respective reactive oxygen scavenger conditions. As such, these two conditions were tested with redox polymer modifications of naphthoquinone (NQ-LPEI) and ferrocene

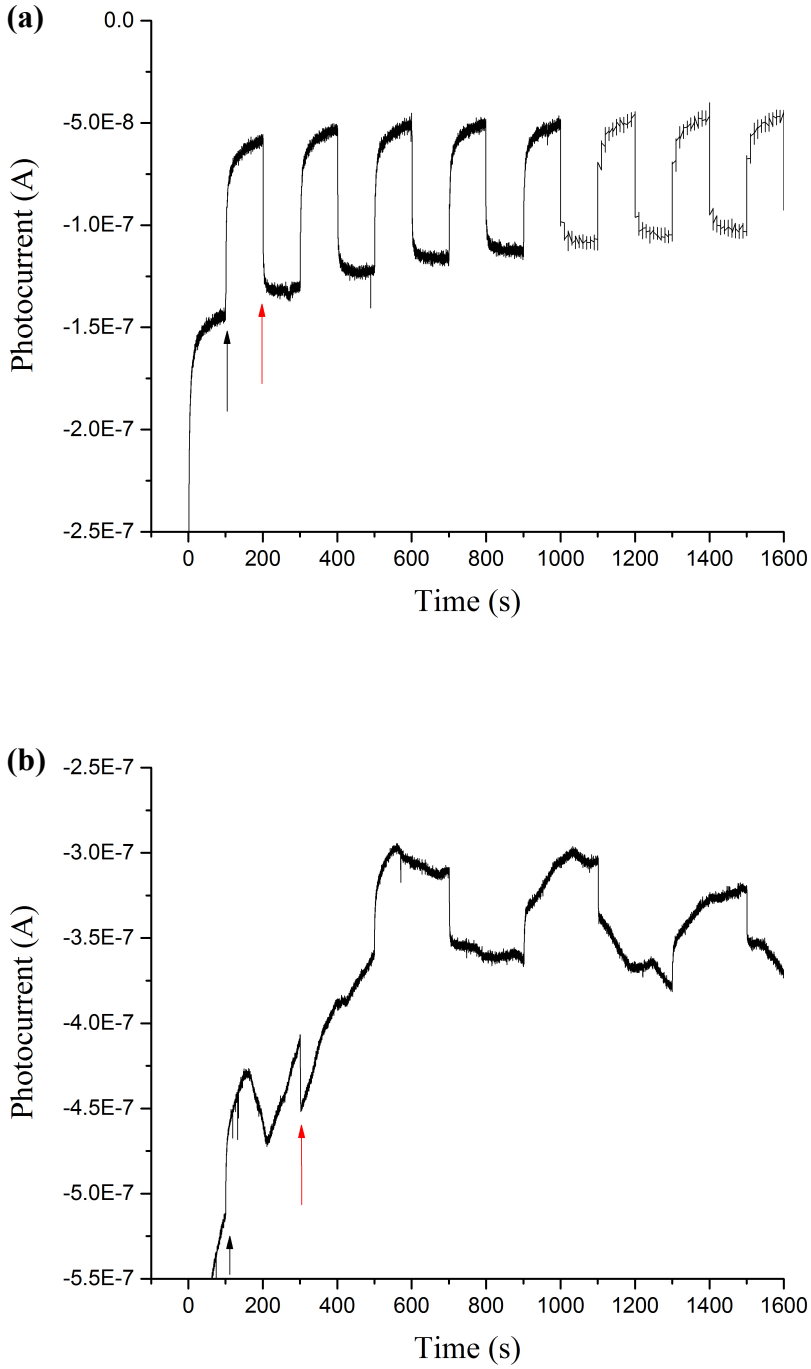

Figure 3-5. Comparison of photostabilization times obtained at 0.45 V vs. saturated Ag/AgCl in 5.5 pH 0.1M citrate buffer for bio-anodes with differing concentrations of ascorbic acid. Black arrows indicate turning the light off, and red arrows indicate turning the light on. (a) Sharp peaks seen every 100 s for 1mM ascorbic acid electrodes. (b) Long 200 s peaks for full stabilization at high 25 mM concentration.

(Fc-LPEI) polymers along with the blank and catalase control bio-anodes.

Additionally, as NQ-LPEI and Fc-LPEI had never been used with thylakoids for photobioelectrocatalysis before, CVs showing proof of photobioelectrocatalytic activity were collected. Images of these CVs can be seen in Figures 3-6 and 3-7.

As can be seen in Figure 3-8, the NQ-LPEI modified blank thylakoid condition produced a significant 183% of the photocurrent seen in the unmodified blank thylakoid anodes. All other NQ-LPEI modified anodes continued to show great photocurrent increases over their respective unmodified conditions, with catalase conditions again producing the lowest currents (greatly increased over unmodified catalase, but not significantly improved over unmodified blank conditions), activated carbon conditions producing middle average values, and ascorbic acid providing the highest photocurrent recorded for the experimental thylakoid anodes at 1.656 ± 0.467 μA/(mg/mL), or 1103% of the unmodified blank thylakoid photocurrent value. While both activated carbon and ascorbic acid NQ-LPEI photocurrents were significantly higher than the blank unmodified photocurrents and had highly separated averages, they were not significantly distinct from one another. Unlike unmodified test conditions, NQ-LPEI modified electrodes also showed higher photocurrents under reactive oxygen scavenger conditions.

For Fc-LPEI modified conditions, as can also be seen in Figure 3-8, the blank condition achieved an average photocurrent 179% of the photocurrent produced by the unmodified blank thylakoid bio-anodes but was not significantly distinct when compared using Student T-tests. Unlike for NQ-LPEI modified electrodes, only Fc-LPEI activated carbon achieved a significantly greater current than unmodified blank anodes, with 235% that of the unmodified blank value, and none of the Fc-LPEI photocurrents were

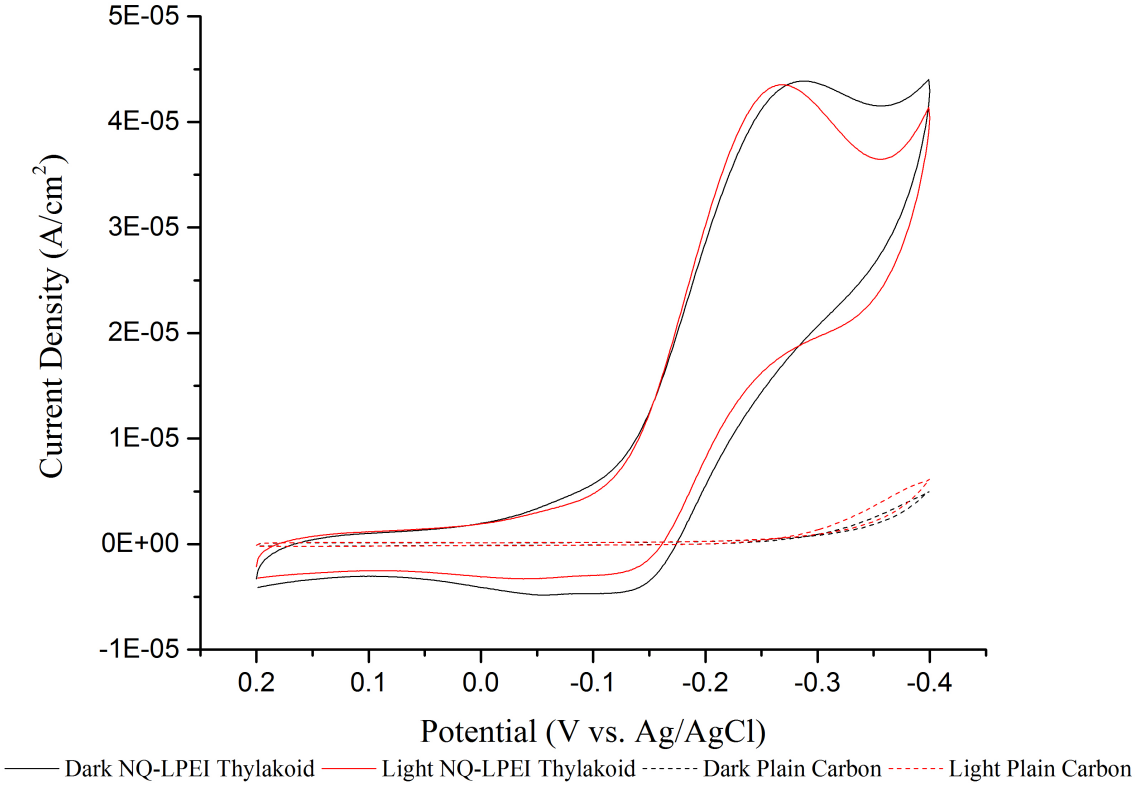

Figure 3-6. Cyclic voltammograms of light and dark NQ-LPEI thylakoid electrode conditions compared to light and dark plain carbon conditions taken at a 0.01 V/s scan rate. The shift towards more negative currents indicates a greater oxidative current produced by the thylakoids in response to light, as expected.

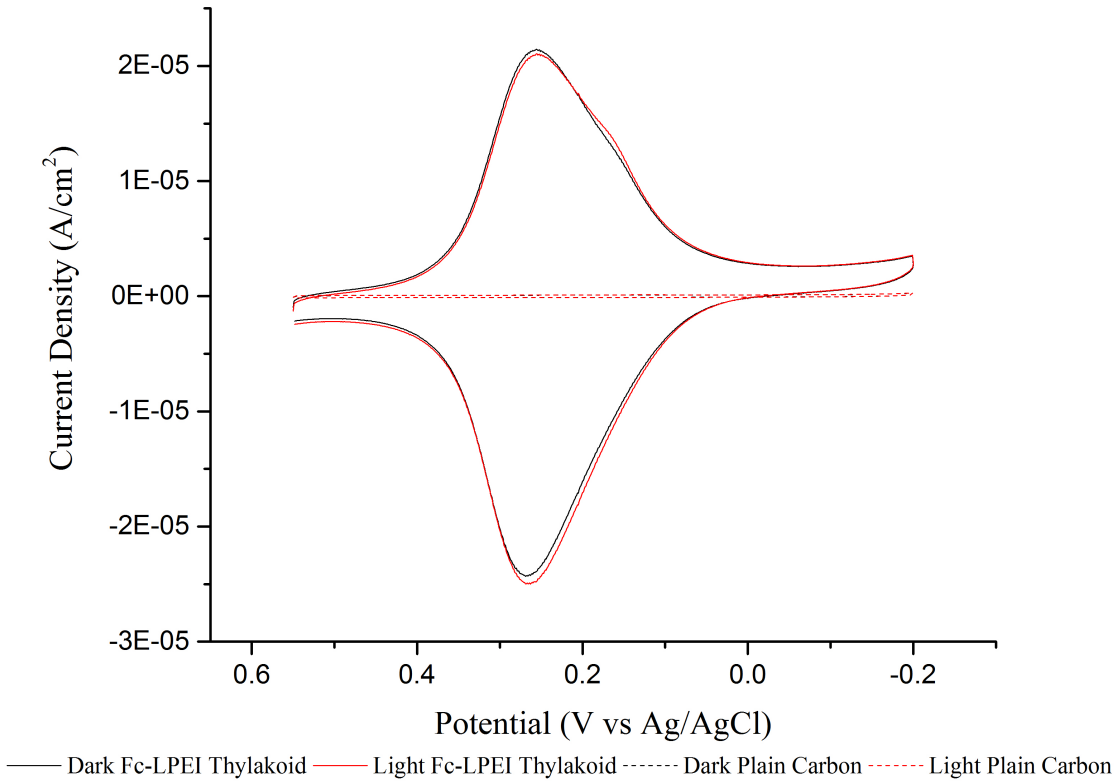

Figure 3-7. Cyclic voltammograms of light and dark Fc-LPEI thylakoid electrode conditions compared to light and dark plain carbon conditions taken at a 0.01 V/s scan rate. The shift towards more negative currents indicates a greater oxidative current produced by the thylakoids in response to light, as expected.

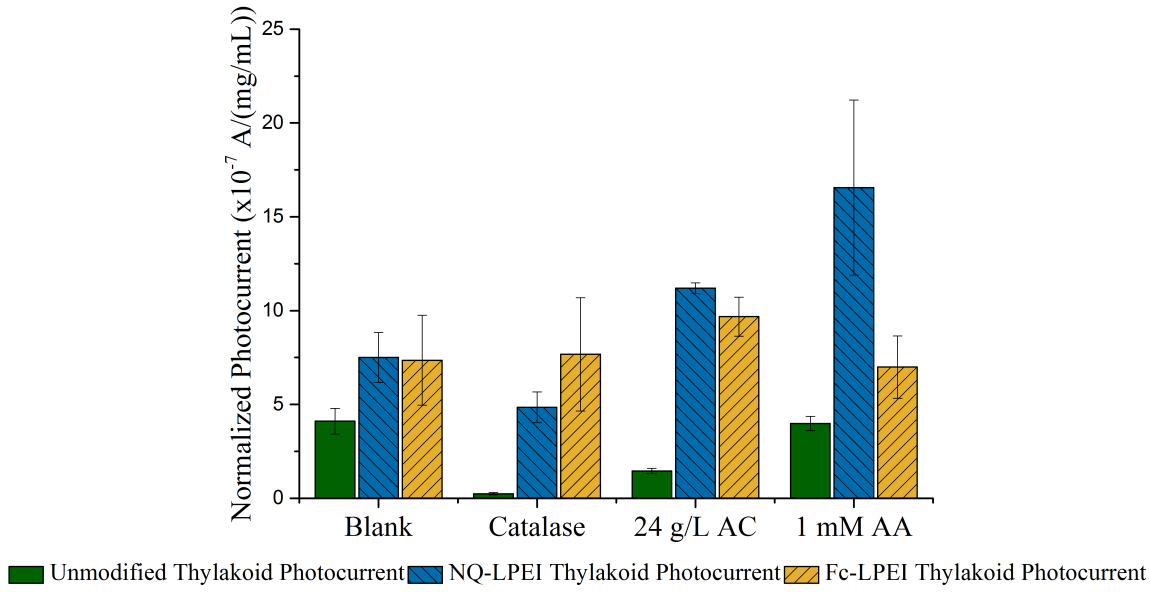

Figure 3-8. Comparison of normalized photocurrents measured for all reactive oxygen scavenger conditions of unmodified, NQ-LPEI modified, and Fc-LPEI modified thylakoid biocatalyst bio-anodes.

significantly distinct from one another.

Additionally, unlike the results for unmodified electrodes, where activated carbon showed the most photocurrent uncertainty of the two experimental reactive oxygen scavenger conditions, both NQ-LPEI and Fc-LPEI anodes had the least uncertainty of photocurrent measurement when activated carbon was present. This suggests that rather than competing with the electrode surface for electrons, as was the case in unmodified anodes, the activated carbon in the modified electrons may be working together with the redox polymer strands to more effectively gather and transport electrons from the thylakoid biocatalyst to the electrode.

Using a literature value for the rate of photosynthesis in spinach chloroplasts of 119 μmol O_2 production/mg chlorophyll/h,[46] estimates of electron transfer efficiency of the electrodes in comparison to unextracted biocatalyst were calculated for each condition. Example calculations can be seen in Appendix B. These efficiency estimates are presented in Table 3-2. Average values and statistical significance follow the same pattern exhibited by the source photocurrent values. Thus, the thylakoid biocatalyst bio-anode which produced the greatest average electron transfer efficiency was the NQ-LPEI immobilized ascorbic acid condition, with an estimated efficiency of 1.437 ± 0.405% at converting light energy into electric current, although this value was not a statistically significant improvement over that of the NQ-LPEI activated carbon electrodes.

To determine whether the increases in photocurrent and corresponding increases in estimated electron transfer efficiency seen for modified electrodes were actually due to the mediating abilities of the redox polymers or simply because the polymer method of immobilization pressed more biocatalyst within DET distance of the electrode surface,

Table 3-2

Electron transfer efficiency estimates for blank, control, activated carbon, ascorbic acid, naphthoquinone redox polymer, and ferrocene redox polymer experimental thylakoid test electrodes.

Electrode Type	Normalized Photocurrent in A/(mg/mL)	Estimated Percent Efficiency (%)
Blank	$4.107 \pm 0.682 \times 10^{-7}$	0.128 ± 0.021
Control (Catalase)	$2.418 \pm 0.647 \times 10^{-8}$	0.007 ± 0.002
3 g/L AC	$3.190 \pm 0.560 \times 10^{-7}$	0.099 ± 0.018
6 g/L AC	$2.677 \pm 0.489 \times 10^{-7}$	0.084 ± 0.015
12 g/L AC	$2.227 \pm 0.402 \times 10^{-7}$	0.069 ± 0.013
24 g/L AC	$1.450 \pm 0.140 \times 10^{-7}$	0.045 ± 0.004
60 g/L AC	$1.763 \pm 0.495 \times 10^{-7}$	0.055 ± 0.015
120 g/L AC	$2.070 \pm 0.849 \times 10^{-7}$	0.065 ± 0.027
1mM AA	$3.986 \pm 0.385 \times 10^{-7}$	0.125 ± 0.012
10 mM AA	$4.357 \pm 0.741 \times 10^{-7}$	0.136 ± 0.023
25 mM AA	$8.192 \pm 5.226 \times 10^{-7}$	0.256 ± 0.163
Blank + NQ-LPEI	$7.503 \pm 1.330 \times 10^{-7}$	0.651 ± 0.115
Control + NQ-LPEI	$4.845 \pm 0.817 \times 10^{-7}$	0.421 ± 0.071
24 g/L AC + NQ-LPEI	$1.120 \pm 0.028 \times 10^{-6}$	0.972 ± 0.025
1mM AA + NQ-LPEI	$1.656 \pm 0.467 \times 10^{-6}$	1.437 ± 0.405
Blank + Fc-LPEI	$7.356 \pm 2.391 \times 10^{-7}$	0.639 ± 0.208
Control + Fc-LPEI	$7.671 \pm 3.016 \times 10^{-7}$	0.666 ± 0.262
24 g/L AC + Fc-LPEI	$9.676 \pm 1.042 \times 10^{-7}$	0.839 ± 0.090
1mM AA + Fc-LPEI	$6.992 \pm 1.836 \times 10^{-7}$	0.607 ± 0.144

blank conditions for unmodified, NQ-LPEI modified, and Fc-LPEI modified thylakoid anodes were compared to a blank electrode immobilized by a C8-LPEI control polymer. Unlike NQ-LPEI and Fc-LPEI, C8-LPEI is not a redox polymer, having only chains of eight carbons positioned periodically along the polymer backbone instead of electroactive functional groups. Thus, if the C8-LPEI control polymer was able to achieve similar levels of photocurrent to the other polymer modified electrodes, the increased photocurrent could be attributed to closer immobilization of thylakoids to the electrode surface rather than actual electron transfer mediation. However, as can be seen in Figure 3-9, the results show that photocurrent for the C8-LPEI electrodes significantly decreases even below that of unmodified electrode values. This suggests that the thylakoids are suspended amidst polymer chains rather than being pressed closely to the electrode surface and that mediation is indeed increasing electron transfer efficiency to the modified electrode surfaces.

However, while modifying thylakoid bio-anodes with redox polymers greatly increased the electron transfer efficiency of the electrodes, a different trend was seen in terms of lifetime and stability. As seen in Figure 3-10, NQ-LPEI and Fc-LPEI modified electrodes showed consistently and significantly lower lifetimes than their respective unmodified reactive oxygen scavenger conditions except in the case of the NQ-LPEI catalase condition, which was comparable to the unmodified catalase condition. The lifetime of the NQ-LPEI blank condition, for example, at 717 ± 115 s, was only 73% of the average lifetime of the unmodified blank electrodes, and that of the Fc-LPEI blank condition, at 433 ± 29 s, was only 44% of the unmodified blank. The presence of reactive oxygen scavengers continued to improve average lifetimes when compared with

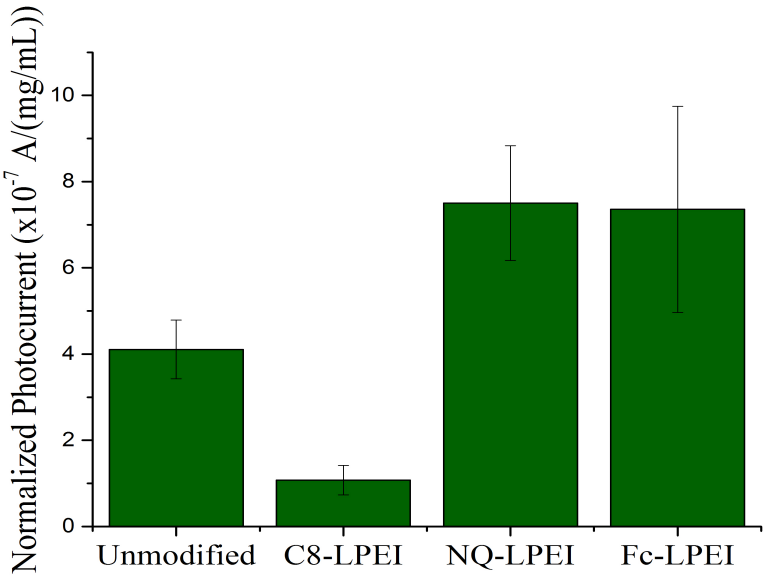

Figure 3-9. Comparison of blank thylakoid unmodified, NQ-LPEI modified, and Fc-LPEI modified photocurrents with that of a control blank C8-LPEI modified condition.

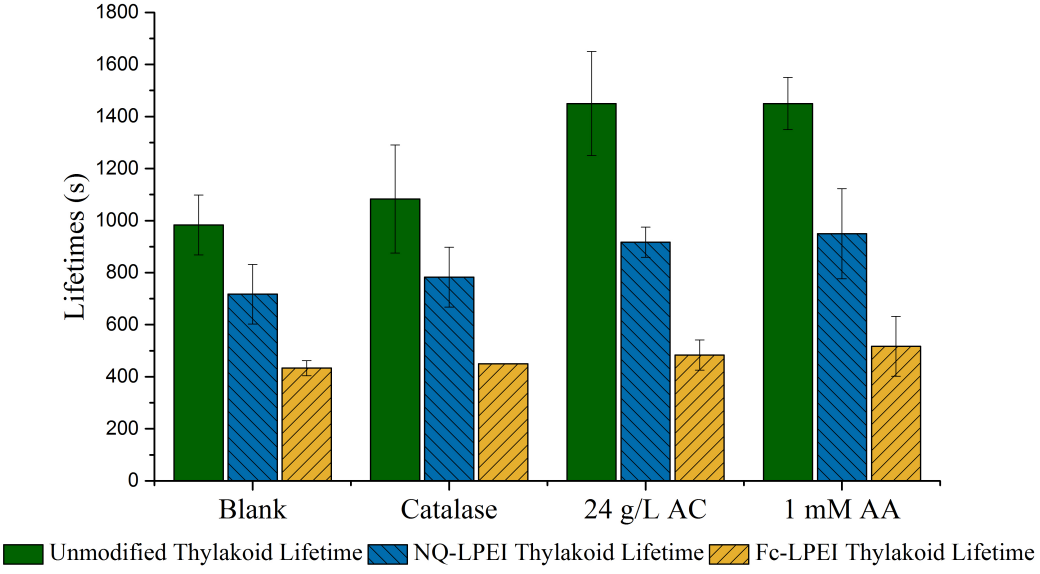

Figure 3-10. Comparison of lifetimes measured for all reactive oxygen scavenger conditions of unmodified, NQ-LPEI modified, and Fc-LPEI modified thylakoid biocatalyst bio-anodes.

the respective immobilized blank value, but with standard deviations considered, no significant differences in lifetimes were seen with the addition of any reactive oxygen species scavenger to either redox polymer immobilized electrode condition.

This trend of decreasing stability of polymer modified electrodes and loss of significant lifetime improvement with added reactive oxygen scavengers suggests that the hydrogel structures of the redox polymers are retaining more reactive oxygen species and immobilizing the thylakoid membranes into folds that trap the photosynthetically produced reactive oxygen species close to the biological structures. This hypothesis seems to be supported by the comparison of thylakoid lifetimes with those immobilized by the C8-LPEI control polymer, another hydrogel, in Figure 3-11, where the C8-LPEI hydrogel shows no significant increase in lifetime over the neighboring NQ-LPEI hydrogel condition.

Summary of Thylakoid Biocatalyst Device Results

For those experimental conditions unmodified by redox polymers, it was concluded that the 1mM ascorbic acid thylakoid bio-anodes created the best combination of lifetime and electron transfer efficiency of the variables tested. This condition increased lifetime to 148% of the blank thylakoid solution, compared to a questionable 110% increase provided by the catalase control, without the photocurrent reduction or high uncertainty seen in catalase and activated carbon measurements.

Both NQ-LPEI and Fc-LPEI modified bio-anodes showed examples of greatly increased electron transfer efficiencies, but with all conditions having greatly decreased biocatalyst stabilities when compared with their respective unmodified conditions. NQ-

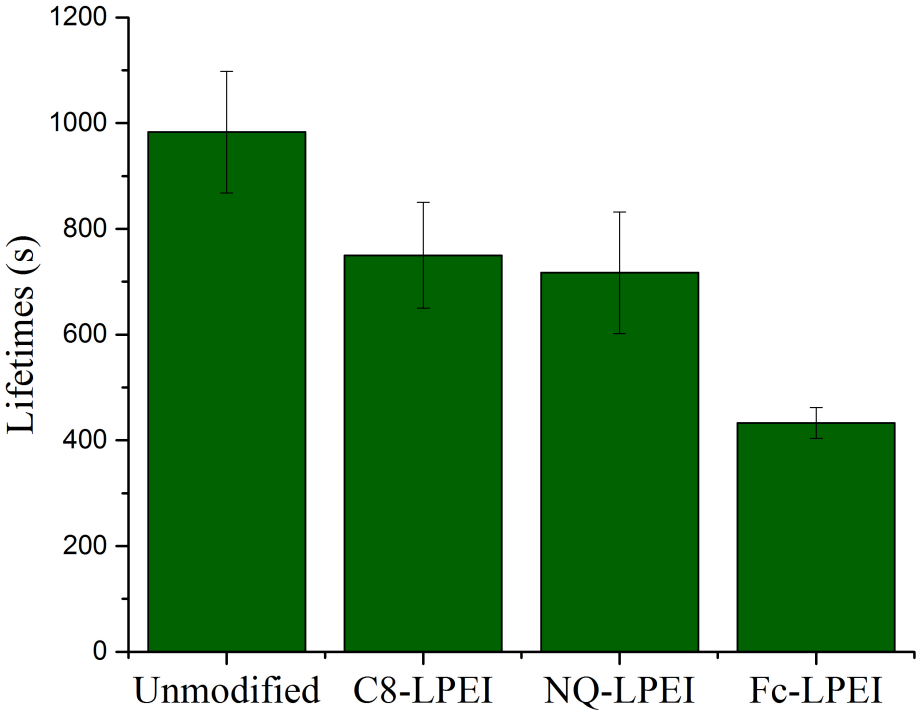

Figure 3-11. Comparison of blank thylakoid unmodified, NQ-LPEI modified, and Fc-LPEI modified lifetimes with that of a control C8-LPEI modified condition.

LPEI blank, activated carbon, and ascorbic acid photocurrents all significantly outperformed their respective unmodified conditions. NQ-LPEI ascorbic acid conditions produced the highest average photocurrent and efficiency achieved out of any thylakoid biocatalyst anode condition, with a value 1103% of the unmodified blank thylakoid photocurrent and an estimated efficiency of 1.437 ± 0.405%, although the NQ-LPEI ascorbic acid values were not significantly distinct from those of the NQ-LPEI activated carbon condition. For Fc-LPEI conditions, only the activated carbon variation showed any significant increase in photocurrent over unmodified blank values. The fact that the NQ-LPEI redox polymer was best able to mediate electrons from thylakoids to the surface of the electrodes was expected due to the naphthoquinone functional groups' ability to transverse membranes and their similarity to the quinone electron acceptors in the PSII electron transport pathways. Poor lifetimes of NQ-LPEI and Fc-LPEI modified anodes may be due to the structure of the hydrogels. These could retain reactive oxygen species and could possibly be immobilizing the thylakoids into unfavorably folded positions that more effectively trap photosynthetically produced reactive oxygen species.

CHAPTER 4

INVESTIGATION OF PHOTOSYSTEM I BIOCATALYST CATHODES

As part of their photosynthetic reactions involve accepting electrons from PSII light reactions, PSI proteins were used to create potential bio-solar cell cathodes. Included within this chapter are all of the relevant procedures used to create and test cathodes made using PSI biocatalysts, followed by a discussion of the collected results. Because many of these procedures are similar to those used to create thylakoid biocatalyst anodes, several procedures will refer to the previous chapter for the bulk of the instructions and list only specific differences in procedure. The sources of materials and compositions of specific buffers mentioned in the following methods can be found in the Appendix A.

Experimental Procedures

Extraction of Photosystem I from Spinach

To begin extraction of PSI from spinach, a centrifuge capable of rotating in excess of 12,000g was precooled to 4 ^{o}C. Solubilization medium buffer was also poured into a flask and incubated in an incubation chamber at 45 ^{o}C. One bunch of spinach was then stripped of its ribs and washed before measuring out 100 grams of wet-weight spinach.

These 100 grams of wet spinach were then blended with 500 mL of chloroplast preparation buffer on the highest setting of a blender for 30 s. The resulting liquid was filtered through four layers of cheesecloth and then centrifuged at 10,000g for 5 min.

The supernatant from this centrifugation was discarded, and the pellets were re-suspended in 10 mL total of chloroplast preparation buffer. No more than 10 mL of buffer was used to prevent over-dilution. The chlorophyll a concentration of the re-suspended pellets was then determined by measuring the absorbance at 665 nm of a cuvette containing 990 μL 80% acetone solution and 10 μL resuspended spinach solution and then multiplying by a dilution factor of 100 before dividing by an extinction coefficient of 90 to obtain mg/mL of chlorophyll a. This chlorophyll determination procedure differs from that used during the thylakoid experiments because the thylakoid calculation accounts for both the concentrations of chlorophyll a and b with its absorbance wavelength and extinction coefficients. In the thylakoid membrane, many accessory chlorophyll molecules and other pigments help to capture and direct light to the reaction centers of the photosystems, where specialized chlorophyll a molecules begin the electron transport reactions. However, once the photosystems are separated from the rest of the thylakoid membrane, only chlorophyll a remains to be found using absorbance measurements.

If the solution was found to have greater than 2 mg/mL of chlorophyll a, it was diluted using chloroplast preparation buffer until it reached this concentration. Two volumes of preheated solubilization medium were then mixed with every one volume of spinach solution. This mixture was then incubated for 30 min at 45 ^{o}C in the dark to prevent photodamage that could occur during the lengthy extraction and purification

process. After incubation, the suspension was chilled in an ice bath for 20 min, again in the dark. Once chilled, the solution was centrifuged at 12,000g for 30 min to remove debris, and the supernatant was then collected for the next step. At this point, if storing the solution overnight to finish the purification process the next day, the supernatant container was wrapped in aluminum foil to occlude light and left in a refrigerator kept at 40 ^{o}F.

An AKTAprime plus Fast Protein Liquid Chromatography, or FPLC, instrument was used to purify the extracted PSI. After inserting Input A of the machine into a container of starting buffer containing no NaCl, and Input B into a container of starting buffer with 200 mM of NaCl included (both containers kept chilled with an icebath), both solutions were run at 5 mL/min to replace the standard ethanol solution within the FPLC tubes. A 5 mL Bio-Rad Bio-Scale™ Mini Macro-Prep® High Q column was then attached, making sure no air bubbles entered between the connections and was washed with 100 mL of starting buffer with no NaCl at 5 mL/min. The supernatant collected from the spinach was then loaded into the column at 1 mL/min until completely absorbed. During this process, both the supernatant container and column were wrapped in aluminum foil to protect against light damage. When the supernatant is absorbed, the input instructions were adjusted so that a combination of the two input tubes allowed a solution of 10 mM NaCl Starting Buffer to flow through the column. This solution was used to wash the column at 1 mL/min until the absorbance readings collected by the FPLC sensors stabilized, around 100 mL. Input instructions were than adjusted again to allow a 50 mL of 50 mM NaCl starting buffer to flow through the machine and column at the same rate.

After this, a 50 mL gradient from 50 mM NaCl to 200 mM NaCl was inputted into the machine, with ten to fifteen continuous 5 mL samples of output being collected in vials that were then stored in a dark icebox as the salt gradient began to pull the purified PSI out of the column. Once the samples were collected, an SDS Page gel electrophoresis test was run for each sample to determine which contained the purified proteins.

While the gel electrophoresis was running, the High Q column was removed, Input A of the FPLC instrument was switched into the equilibration buffer, and Input B was switched into the elution buffer. These solutions were also kept cool by an icebath. A hydroxyapatite column was connected in place of the High Q column and washed with equilibration buffer at 1 mL/min. At this point, the collected samples which showed characteristic PSI protein patterns via the SDS Page gels were diluted with an equal volume of hydroxyapatite medium and loaded into the new column. This was then washed with 20 mL of equilibration buffer at 1 mL/min. Finally, after eluting with elution buffer until the absorbance readings of the FPLC begin to stabilize in order to collect a concentrated preparation of ultrapure PSI, 20% of sucrose was added to the final amount.

Storage

The sugar-supported PSI was then immediately divided into 0.5 mL Eppendorf microcentrifuge tubes. These were flash frozen with liquid nitrogen to prevent the damage gradual freezing could cause to the delicate protein fragments before being stored in a freezer at -80 ^{o}C. The PSI could then be unfrozen when convenient and used for

several months.

Preparation of Cathodes Without Incorporating Redox Polymers

PSI bio-cathodes were prepared in much the same way as the thylakoid bio-anodes described early. The only differences were that, in order to preserve the supply of very labor-intensively extracted PSI for as long as possible, much smaller volumes of biocatalyst were thawed out and modified at a time, and rather than measuring for both chlorophyll a and b when determining initial chlorophyll concentration, the wavelength and equation modified for chlorophyll a only were used. Only the optimal concentrations of reactive oxygen scavengers determined during initial thylakoid testing were used for PSI testing.

Preparation of Cathodes Incorporating Redox Polymers

The procedures to prepare PSI bio-cathodes incorporating redox polymers were also very similar to those used for the thylakoid redox polymer anodes. Again, biocatalyst volumes were thawed and used more conservatively and characterized for chlorophyll concentration using the modified chlorophyll a technique.

Amperometric Testing

An identical setup and amperometric testing procedure was used for both PSI biocatalyst and thylakoid biocatalyst electrodes except that tests were run at a potential of -0.1 V vs. saturated Ag/AgCl. Electrodes were still illuminated for 300 s prior to testing. Photocurrent, determined by the difference between the light off and light on conditions

of the second current peak, was still monitored until 25% photocurrent reduction. For PSI tests, only the previously biocatalyst-optimized concentrations of reactive oxygen scavengers were tested.

Cyclic Voltammetry

Cyclic voltammetry was also conducted identically for the PSI cathodes, except that a biocatalyst condition unmodified by redox polymer was also tested. Blank silica immobilized PSI, NQ-LPEI immobilized PSI, and Fc-LPEI immobilized PSI were tested. As PSI is a reductive biocatalyst, shifts towards more positive currents indicate a greater reductive current produced by the system in the presence of light were expected, in contrast to the negative oxidative currents expected for thylakoids.

Results and Discussion

As photobioelectrocatalysis using PSI and PSI NQ-LPEI/Fc-LPEI modified systems had not been attempted within the lab before, CVs were conducted for these systems as proofs of photobioelectrocatalytic activity. They can be seen in Figures 4-1, 4-2, and 4-3. Amperometric results were also collected and analyzed for each system. Table 4-1 summarizes the amperometric results collected. As before, "Lifetime" refers to the amount of time it took to reach 25% reduction in initial photocurrent, and "Normalized Photocurrent" refers to the fact that each photocurrent has been normalized by the concentration of chlorophyll in mg/mL found for the PSI solutions that made each batch of electrodes. Modified electrodes were again also normalized by biocatalyst volume, as well. Abbreviations include AC, meaning activated carbon; AA, meaning

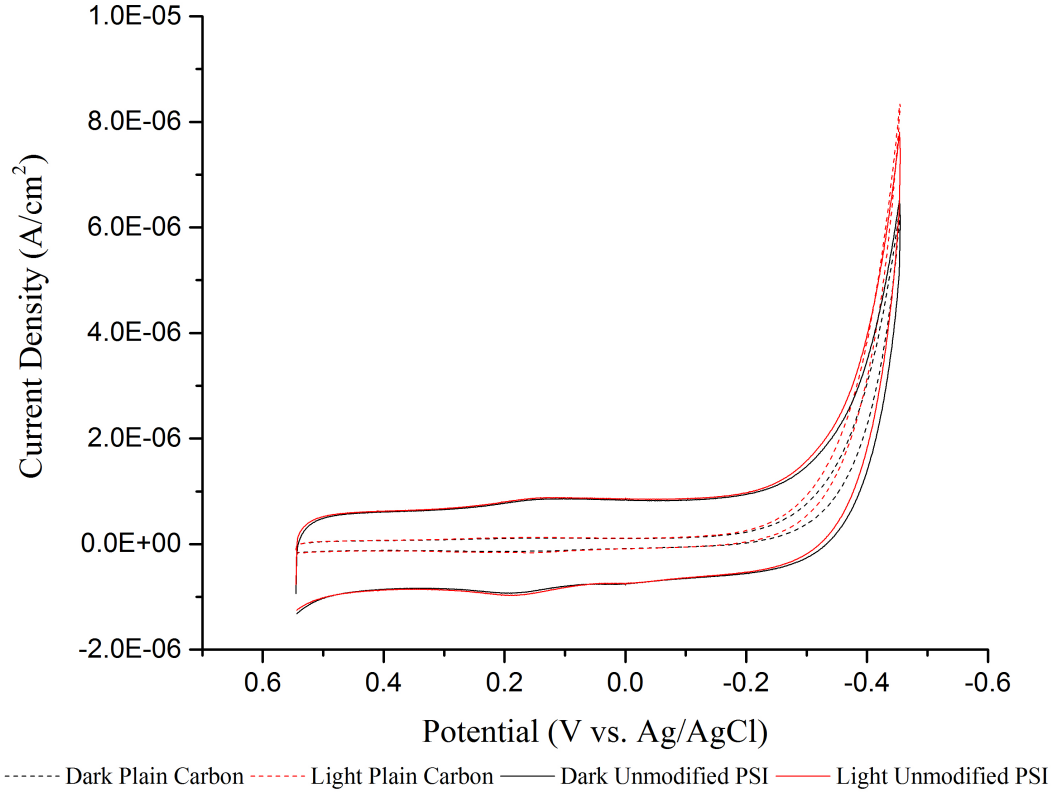

Figure 4-1. Long scan cyclic voltammograms of light and dark unmodified PSI electrode conditions compared to light and dark plain carbon conditions run at a 0.01 V/s scan rate. As can be seen, there is not much improvement over the plain carbon electrode, although the red lighted conditions do show a more reductive current than dark, as expected.

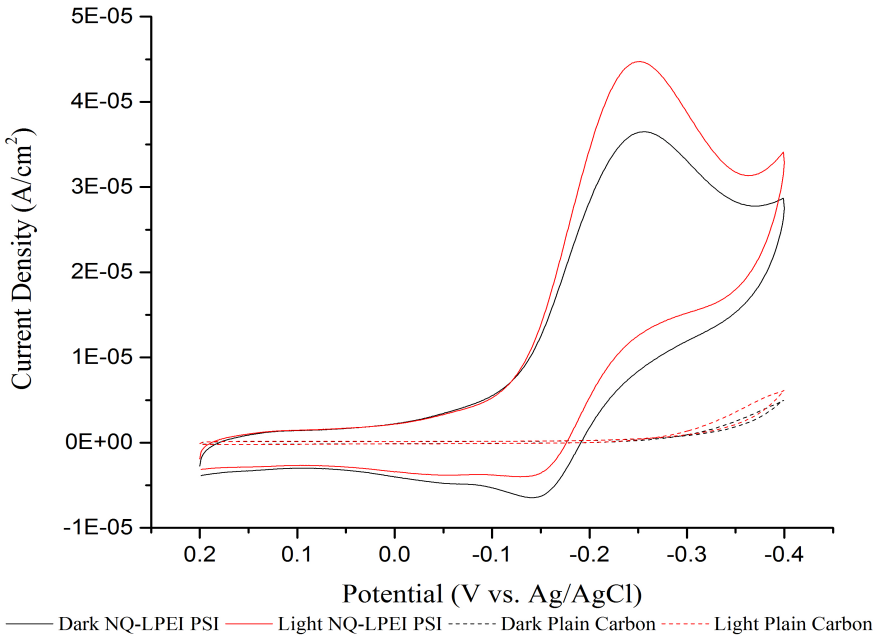

Figure 4-2. Cyclic voltammograms of NQ-LPEI PSI electrode conditions compared to plain carbon conditions run at a 0.01 V/s scan rate. This CV that shows a recognizable photobioelectrocatalytic response with increased reductive light currents, as expected.

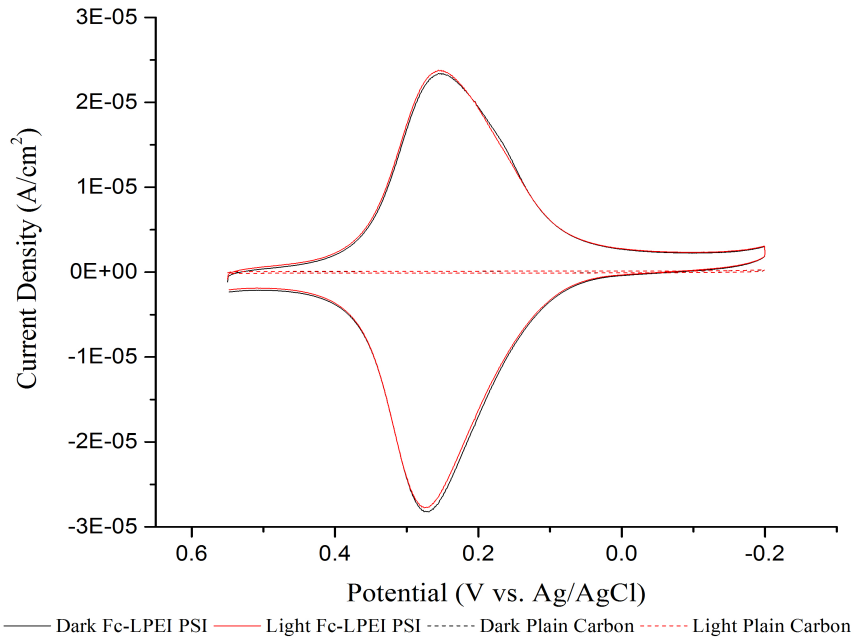

Figure 4-3. 0.01 V/s scan rate cyclic voltammograms of Fc-LPEI PSI electrode compared to plain carbon conditions. Slight increase in light current also seen.

Table 4-1

Amperometric results of blank, control, activated carbon, ascorbic acid, naphthoquinone redox polymer, and ferrocene redox polymer experimental photosystem I test electrodes.

Electrode Type	Lifetime in Seconds	Normalized Photocurrent in A/(mg/mL)
Blank	1050 ± 173	$1.082 \pm 0.188 \times 10^{-7}$
Control (Catalase)	883 ± 58	$2.161 \pm 0.489 \times 10^{-7}$
24 g/L AC	1150 ± 173	$2.826 \pm 0.613 \times 10^{-7}$
1mM AA	1083 ± 58	$4.159 \pm 0.449 \times 10^{-8}$
Blank + NQ-LPEI	983 ± 58	$3.022 \pm 0.574 \times 10^{-6}$
Control + NQ-LPEI	1650 ± 100	$3.717 \pm 0.749 \times 10^{-6}$
24 g/L AC + NQ-LPEI	1416 ± 153	$2.456 \pm 0.566 \times 10^{-6}$
1mM AA + NQ-LPEI	1050 ± 100	$1.420 \pm 0.221 \times 10^{-6}$
Blank + Fc-LPEI	2083 ± 225	$5.429 \pm 0.196 \times 10^{-6}$
Control + Fc-LPEI	2600 ± 200	$5.642 \pm 1.033 \times 10^{-6}$
24 g/L AC + Fc-LPEI	2300 ± 173	$4.424 \pm 0.444 \times 10^{-6}$
1mM AA + Fc-LPEI	1733 ± 58	$5.232 \pm 0.747 \times 10^{-6}$

ascorbic acid; NQ-LPEI, meaning naphthoquinone modified linear polyethyleneimine; and Fc-LPEI, meaning ferrocene modified linear polyethyleneimine.

Examples of Raw PSI Amperometric Data

Unlike thylakoid bio-anodes, which exhibit a more negative oxidative current in the presence of light, the PSI bio-cathodes showed more positive reductive currents upon illumination. This can be seen in Figure 4-4, the raw amperometric data for a blank, unmodified PSI anode. As the reactive oxygen species scavenger concentrations optimized for thylakoids were used for PSI test electrodes, few additional observations were made based on the raw amperometric data. However, it is notable that the raw amperometric data for the blank activated carbon conditions, as seen in Figure 4-5, still show evidence of the increased noise seen for the thylakoid activated carbon conditions. This suggests that the activated carbon particles continued to provide competing electron pathways for the photosynthetic electrons which were captured for the PSI biocatalyst, as they did for the thylakoid biocatalyst.

Unmodified Cathodes

When comparing blank, unmodified PSI electrodes to their equivalent thylakoid condition, as shown in Figure 4-6, the lifetimes of both PSI and thylakoid electrodes are actually comparable with no significant difference for any treatments other than the respective ascorbic acid conditions, despite the supposedly increased fragility of the isolated PSI proteins. This suggests that the lack of native reactive oxygen species being produced by this particular photosystem may balance out any other durability problems it

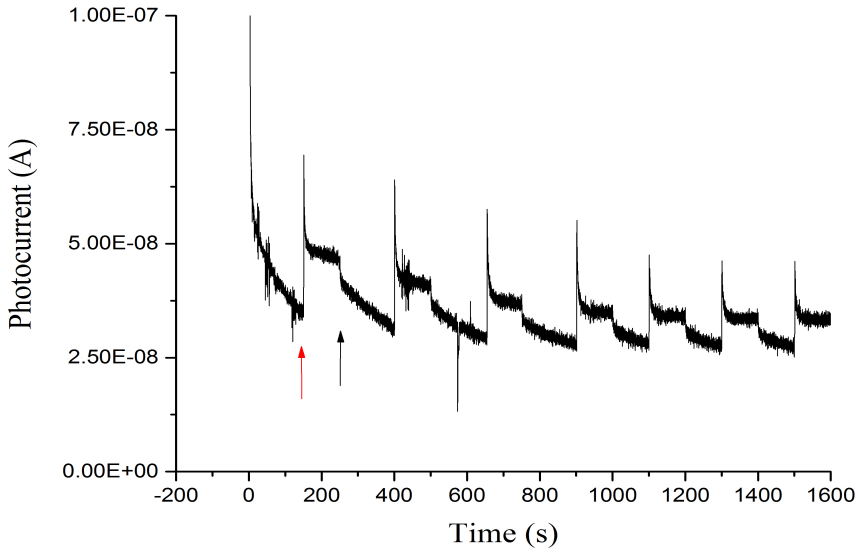

Figure 4-4. Raw amperometric data for an unmodified blank PSI anode obtained at -0.1 V vs. saturated Ag/AgCl in 5.5 pH 0.1M citrate buffer. The red arrow indicates turning the light on and the black arrow indicates turning the light off.

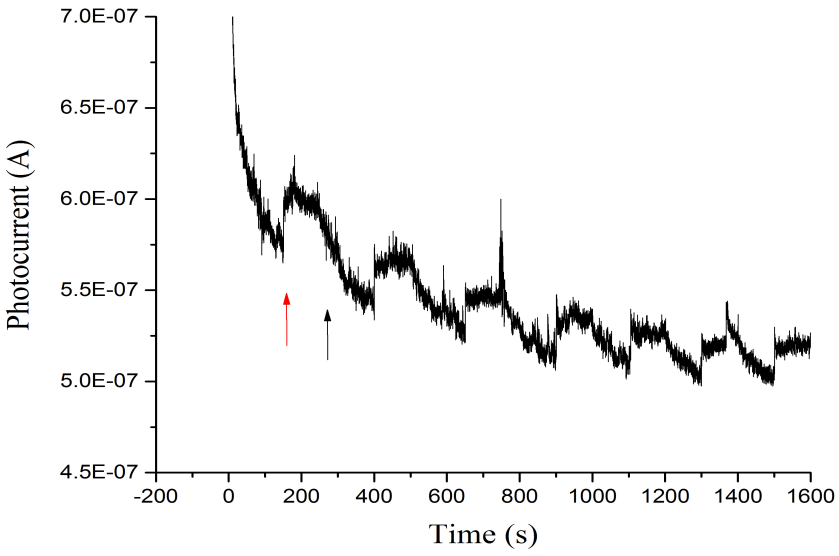

Figure 4-5. Raw amperometric data for an unmodified activated carbon PSI anode obtained at -0.1 V vs. saturated Ag/AgCl in 5.5 pH 0.1M citrate buffer, demonstrating the noisiest data conditions seen. The red arrow indicates turning on the light, and the black arrow indicates turning off the light.

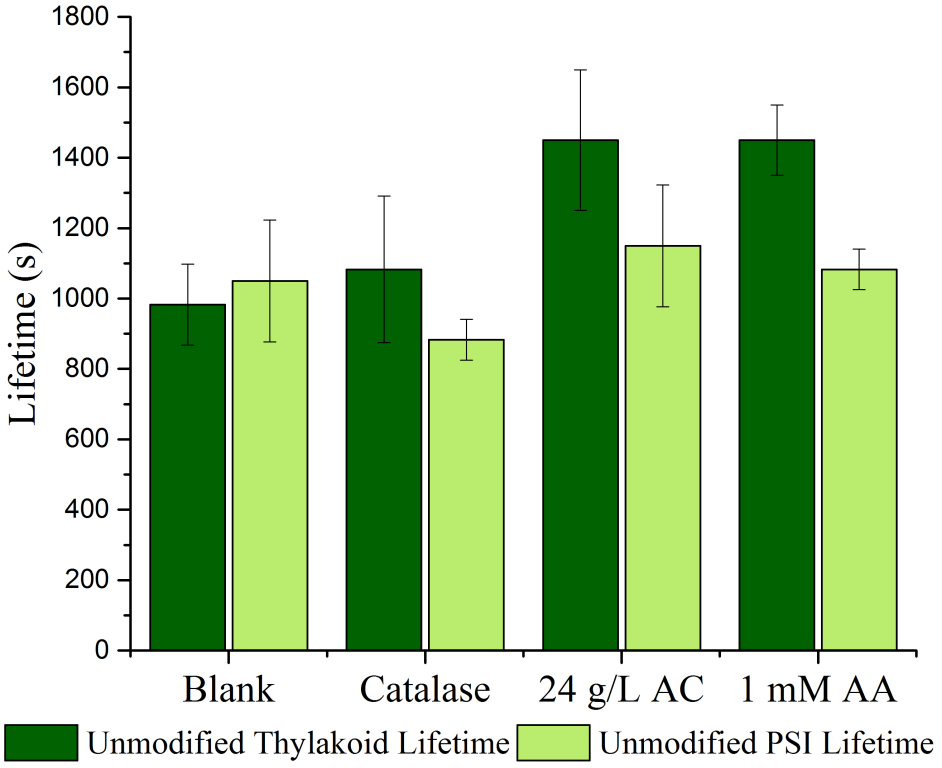

Figure 4-6. Comparison of lifetimes measured for all reactive oxygen scavenger conditions of unmodified PSI bio-electrodes with the corresponding reactive oxygen scavenger conditions of unmodified thylakoid bio-electrodes.

may have compared to the more physically strong thylakoid membranes. This theory is further supported by the fact that the lifetimes of unmodified PSI bio-cathodes shown in Figure 4-6 show no consistent or significant improvement with added reactive oxygen scavengers and are statistically comparable to their corresponding scavenger supported thylakoid electrode conditions except in the case of the ascorbic acid conditions.

As seen in Figure 4-7, when compared to the unmodified thylakoid electrodes in terms of photocurrent, the collected data suggest that in the unmodified, silica bound state, PSI has no reliably greater DET properties than thylakoid membranes, which is also contrary to hypothetical expectations. Although both catalase and activated carbon unmodified PSI photocurrents were statistically greater than their respective unmodified thylakoid conditions, the blank and ascorbic acid unmodified PSI photocurrents were also significantly lower. Of the unmodified PSI electrode conditions, activated carbon produces the greatest average photocurrent, at $2.826 \pm 0.613 \times 10^{-7}$ A/(mg/mL), although this is not significantly greater than the catalase unmodified PSI photocurrent. This suggests that activated carbon may be more effective at extracting electrons from the isolated proteins and transferring them to the electrode surface than from complicated, folded thylakoid membranes, despite continuing to provide competing electron pathways. It is also noticeable within Figure 4-7 that the ascorbic acid condition of the unmodified PSI electrodes has significantly less photocurrent than any of the other conditions at $4.159 \pm 0.449 \times 10^{-8}$ A/(mg/mL). This suggests that in the absence of environments with extra oxygen species to engage it, the acidity of ascorbic acid may be causing the proteins to denature into configurations that take the active sites of the photosynthetic proteins further from the electrode surface, inhibiting electron transfer.

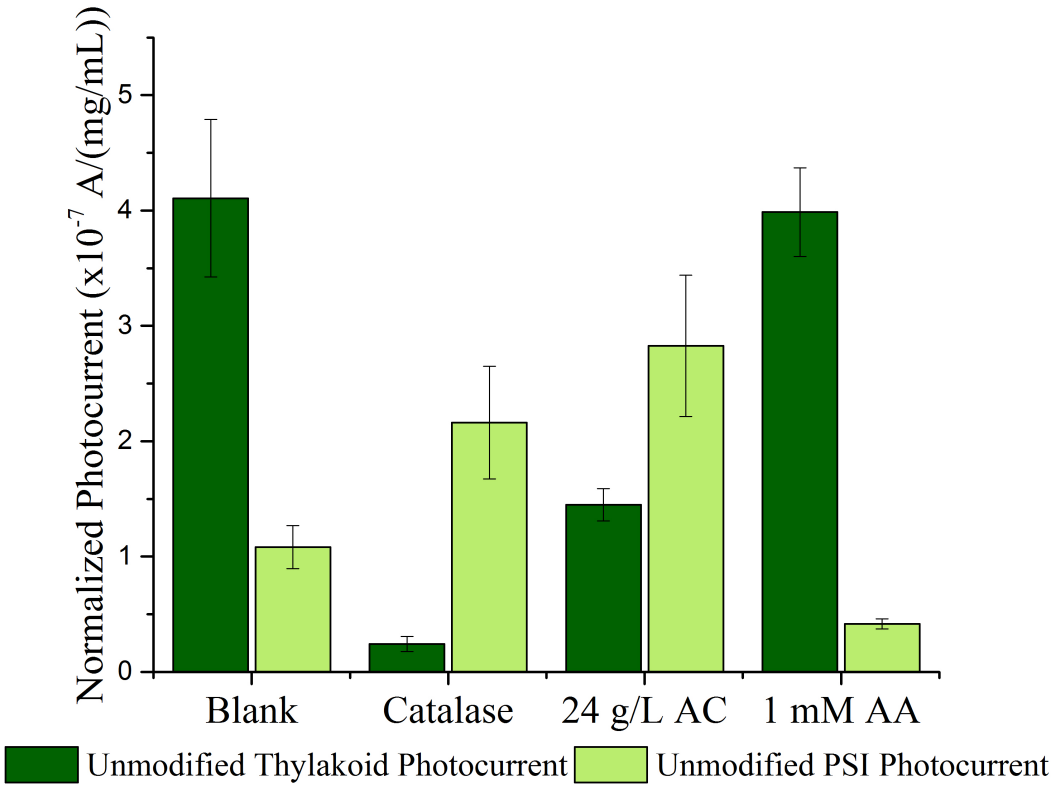

Figure 4-7. Comparison of photocurrents measured for all the reactive oxygen scavenger conditions of unmodified PSI bio-electrodes with the corresponding reactive oxygen scavenger conditions of unmodified thylakoid bio-electrodes.

Redox Polymer Cathodes

The addition of redox polymer, as can be seen in Figure 4-8, greatly improves electron transfer efficiency over the unmodified, silica-immobilized PSI conditions, however. NQ-LPEI immobilized PSI was able to significantly increase blank PSI photocurrent to 2792% of the blank unmodified PSI (equivalent to 735% of the unmodified blank thylakoid photocurrent) with a value of 3.022 ± 0.574 μA/(mg/mL). Blank Fc-LPEI electrodes were also able to significantly increase average photocurrent value to 5017% of blank unmodified PSI (1321% of blank thylakoid condition) with an average photocurrent of 5.429 ± 0.196 μA/(mg/mL). For both NQ-LPEI and Fc-LPEI conditions, catalase conditions yielded the highest average photocurrents (although neither of these were statistically significant from their respective NQ-LPEI and Fc-LPEI blank photocurrents), with the Fc-LPEI catalase condition producing 5.642 ± 1.033 μA/(mg/mL). This was the highest experimental photocurrent measured for any combination of biocatalyst, reactive oxygen scavenger, and immobilization method (1375% of unmodified blank thylakoid condition). Of all the redox polymer immobilized conditions, activated carbon conditions provided the only significantly lower photocurrent for Fc-LPEI modified electrodes from the original blank Fc-LPEI value, suggesting that direct contact between the photosystems and redox polymer is a more desirable way to transfer electrons. Ascorbic acid conditions provided the only significantly lower photocurrent from beginning blank photocurrent for the NQ-LPEI modified electrodes.

With the same literature value for the rate of photosynthesis in spinach chloroplasts used in calculations for the thylakoid bio-anodes, estimates of electron

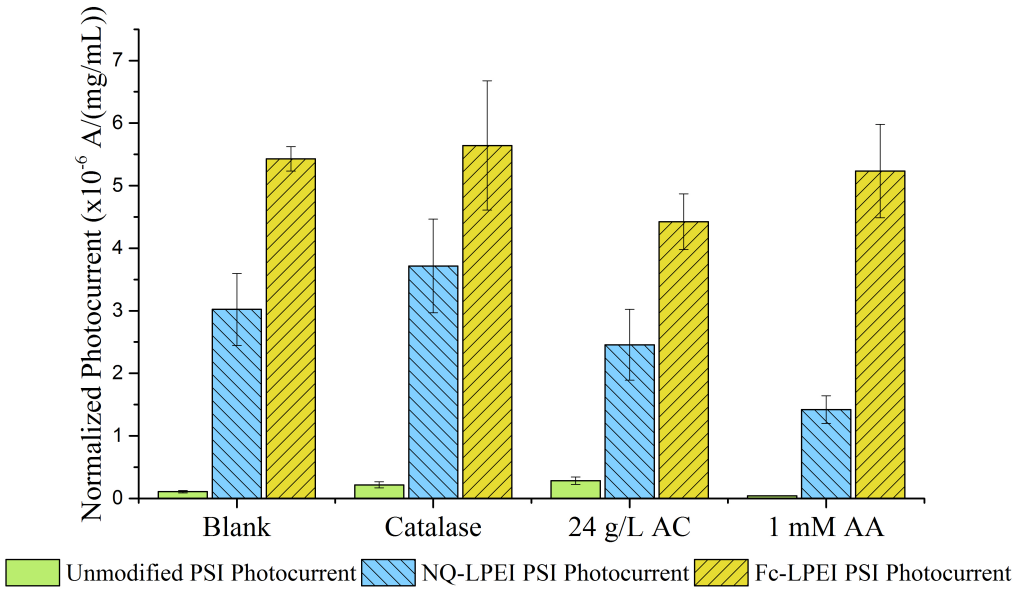

Figure 4-8. Comparison of normalized photocurrents for all reactive oxygen scavenger conditions for unmodified, NQ-LPEI modified, and Fc-LPEI modified PSI biocatalyst bio-cathodes.

transfer efficiency of the electrodes in comparison to unextracted biocatalyst were calculated for each condition. Example calculations can again be seen in Appendix B. These efficiency estimates are presented in Table 4-2. Average values and statistical significance follow the same pattern exhibited by the source photocurrent values. Thus, both NQ-LPEI and Fc-LPEI modified PSI electrodes are again shown to have significant increased electron transfer efficiencies over both unmodified PSI and unmodified thylakoid electrodes. As an immobilization category, the Fc-LPEI modified electrodes show the highest average estimated efficiencies with ranges between 3.841 ± 0.385% for the activated carbon condition and 4.898 ± 0.896% for the catalase condition, although none of the Fc-LPEI blank, catalase, and ascorbic acid conditions are statistically significant from each other.

To determine whether the increases in photocurrent and electron transfer efficiency were due to effective redox polymer mediation or immobilization of more biocatalyst closer to the electrode surface, blank results for unmodified, NQ-LPEI immobilized, and Fc-LPEI immobilized conditions were again compared to a blank sample of biocatalyst, this time PSI, immobilized by the control C8-LPEI polymer, whose nonelectroactive functional groups would prevent any photocurrent increase from occurring due to mediation. As shown in Figure 4-9, it appears that the higher photocurrents from polymer immobilized electrodes may be a result of both mechanisms. C8-LPEI, at a photocurrent of $5.729 \pm 0.618 \times 10^{-7}$ A/(mg/ml), was significantly higher than that of unmodified silica, suggesting more PSI was immobilized within DET distance of the electrode surface in the polymer immobilized electrodes. However, the C8-LPEI current was also significantly smaller than either redox polymer immobilized

Table 4-2

Estimated electron transfer efficiencies of blank, control, activated carbon, ascorbic acid, naphthoquinone redox polymer, and ferrocene redox polymer experimental photosystem I test electrodes.

Electrode Type	Normalized Photocurrent in A/(mg/mL)	Estimated Percent Efficiency (%)
Blank	$1.082 \pm 0.188 \times 10^{-7}$	0.034 ± 0.006
Control (Catalase)	$2.161 \pm 0.489 \times 10^{-7}$	0.068 ± 0.015
24 g/L AC	$2.826 \pm 0.613 \times 10^{-7}$	0.088 ± 0.019
1mM AA	$4.159 \pm 0.449 \times 10^{-8}$	0.012 ± 0.001
Blank + NQ-LPEI	$3.022 \pm 0.574 \times 10^{-6}$	2.623 ± 0.498
Control + NQ-LPEI	$3.717 \pm 0.749 \times 10^{-6}$	3.226 ± 0.651
24 g/L AC + NQ-LPEI	$2.456 \pm 0.566 \times 10^{-6}$	2.132 ± 0.492
1mM AA + NQ-LPEI	$1.420 \pm 0.221 \times 10^{-6}$	1.232 ± 0.192
Blank + Fc-LPEI	$5.429 \pm 0.196 \times 10^{-6}$	4.713 ± 0.170
Control + Fc-LPEI	$5.642 \pm 1.033 \times 10^{-6}$	4.898 ± 0.896
24 g/L AC + Fc-LPEI	$4.424 \pm 0.444 \times 10^{-6}$	3.841 ± 0.385
1mM AA + Fc-LPEI	$5.232 \pm 0.747 \times 10^{-6}$	4.542 ± 0.648

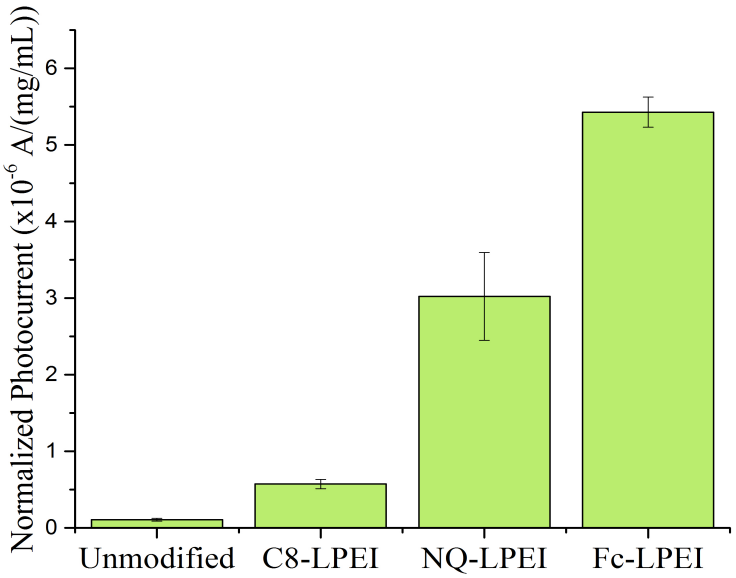

Figure 4-9. Comparison of blank PSI unmodified, NQ-LPEI modified, and Fc-LPEI modified photocurrents with that of a control polymer C8-LPEI modified condition.

photocurrent, indicating that there was still a great increase in electron transfer efficiency between easily reachable protein active sites and the redox polymer functional groups.

In terms of stability, unlike the results seen for the thylakoid anodes, which showed great decreases in lifetime upon redox polymer immobilization, modified PSI cathode lifetimes were comparable or increased even above those of the highest performing thylakoid electrodes. As seen in Figure 4-10, the catalase conditions produced the longest lifetimes for both NQ-LPEI and Fc-LPEI immobilized cathodes, with NQ- LPEI achieving a significant lifetime increase to 1650 ± 100 s (157% of unmodified blank PSI lifetime and 168% of unmodified blank thylakoid), and Fc-LPEI showing an even greater significant increase to 2600 ± 200 s (248% of unmodified blank PSI and 264% of unmodified blank thylakoid).

The much greater increases in lifetime for PSI polymer immobilized electrodes suggests that protein stability benefits not only from lower native production of oxygen, but also from close linking and immobilization by the polymer strands. This seems to be supported by Figure 4-11, which compares the lifetimes of blank PSI unmodified, NQ-LPEI modified, and Fc-LPEI modified conditions with a blank PSI C8-LPEI condition. The lifetimes for the blank polymer immobilized electrodes are all comparable, or significantly greater in the case of Fc-LPEI, than that of the unmodified blank PSI only isolated from outside sources of reactive oxygen species.

Additionally, while catalase is consistently the longest lasting average condition for modified electrodes, it is not significant from either NQ-LPEI or Fc-LPEI respective activated carbon conditions. Ascorbic acid, contrary to the beneficial lifetime effects shown in thylakoid electrodes, had the lowest reactive oxygen scavenger condition

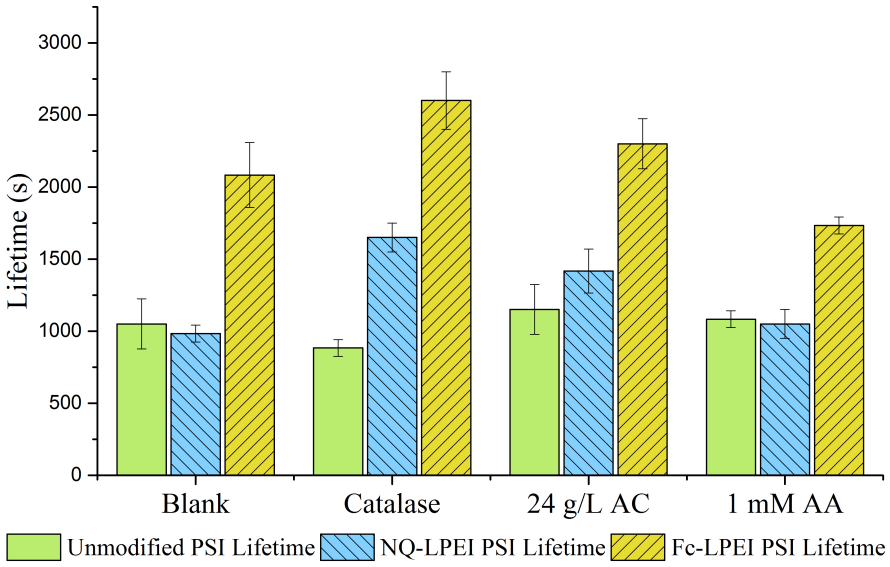

Figure 4-10. Comparison of lifetimes for all reactive oxygen scavenger conditions for unmodified, NQ-LPEI modified, and Fc-LPEI modified PSI biocatalyst bio-cathodes.

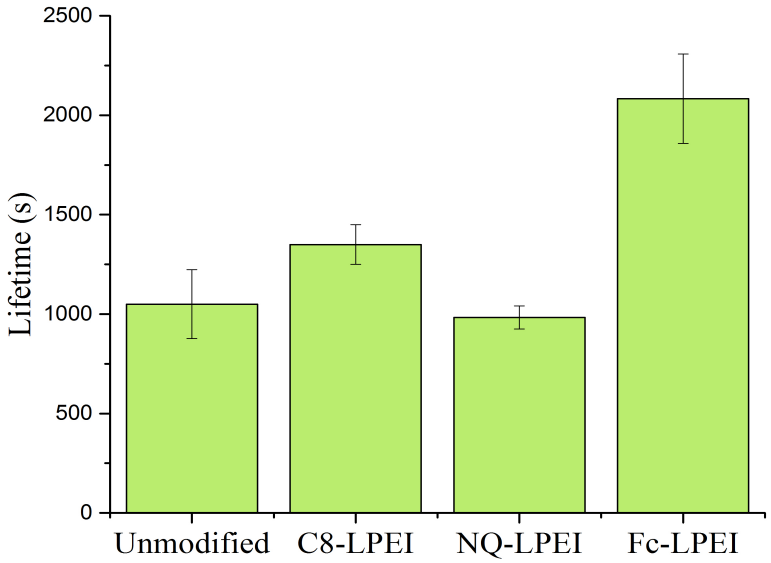

Figure 4-11. Comparison of blank PSI unmodified, NQ-LPEI modified, and Fc-LPEI modified lifetimes with that of a control polymer C8-LPEI modified condition.

average lifetimes, with results of the Fc-LPEI immobilized ascorbic acid PSI actually lasting significantly less long (1733 ± 58 s) than the Fc-LPEI immobilized blank PSI condition (2083 ± 225 s). This suggests once again that the slight change in acidity may be interfering with the polymer structure or the photosystem proteins themselves.

Summary of Photosystem I Biocatalyst Device Results

Unmodified PSI bio-cathodes showed unexpectedly long lifetimes and no consistent evidence of increased DET with the electrode surface when compared with unmodifiedthylakoid electrode results, contrary to hypothetical expectations. It is believed that the long lifetimes of the silica immobilized PSI despite its more fragile nature as isolated proteins is due to the fact that PSI creates no oxygen during its photosynthetic operations, unlike thylakoid membranes, and so does not trap any reactive oxygen species that could deteriorate the biocatalyst within the electrode system.

Once the PSI biocatalyst was immobilized with redox polymer, it showed both significant increases in electron transfer efficiency and an increase in lifetime for most conditions when compared to blank unmodified PSI values. Fc-LPEI catalase PSI cathodes showed the highest average photocurrent of 5.642 ± 1.033 x 10^{-6} A/(mg/mL), estimated efficiency of 4.898 ± 0.896% (although neither of these values were significant from those of other Fc-LPEI conditions), and lifetime of 2600 ± 200 s (which was not significantly distinct from the Fc-LPEI activated carbon condition) recorded for any bio-electrode conditions tested for both biocatalysts. Both increased DET due to closer immobilization of PSI to the electrode surface by the polymer as well as successful MET of electrons from the biocatalyst by the redox functional groups helped to provide these

increases in electron transfer efficiency. The success of Fc-LPEI in particular of mediating PSI was expected both due to the similarity of the iron complex redox functional groups to the iron-sulfur complexes that are part of the PSI electron transport chain and its more favorable redox potential. The great increase in lifetime seen for catalase conditions of both polymers is attributed not only to the lack of native oxygen byproducts of photosynthesis, but also due to more beneficial immobilization of PSI by the redox polymers than occurred for the thylakoid biocatalyst. In terms of reactive oxygen scavenger performance, no significant evidence of consistent lifetime increase with scavenger addition were observed. While catalase was the most successful reactive oxygen scavenger additive tested for modified PSI conditions in terms of average lifetime, it was not significantly different from activated carbon, which showed signs of preventing the best protein/polymer mediation and stabilization, and ascorbic acid results suggested this additive may have been damaging both PSI protein and polymer structures.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The goal of this project was to discover whether reactive oxygen species scavengers other than catalase and application of redox polymer immobilization might increase the lifetimes and electron transfer efficiency of thylakoid and photosystem I biocatalyst electrodes above the current performance standard in hopes of furthering progress towards a cheap, clean, and effective bio-solar cell.

In terms of biocatalyst, thylakoids were expected to have greater inherent stability (increased lifetime) and more difficulty establishing DET with the electrode surfaces (decreased electron transfer electron efficiency). They oxidize water as part of their photosynthetic process and produce potentially reactive oxygen species that may damage the biological components of the electrodes. This oxidation reaction makes them more suited for possible use as bio-solar cell anode biocatalyst. Isolated PSI protein biocatalysts were expected to have lower stability (decreased lifetime) but greater ease at establishing DET (increased electron transfer efficiency). They accept electrons from PSII during their photosynthetic reactions and do not produce any reactive oxygen species, which was seen as a favorable characteristic for an isolated biocatalyst. Due to this reduction reaction of accepting electrons, PSI biocatalysts are more suited for use as

part of a bio-solar cell cathode.

For experimental reactive oxygen scavengers in addition to catalase, ascorbic acid was chosen for testing based on ample evidence of its oxygen scavenging ability and beneficial effects in plant biology. Activated carbon was chosen as a material that, having not been studied extensively, had theoretical cases made for either improving or worsening device performance. Namely, it was proposed that the high surface area and conductive nature of the carbon particles might improve DET with the biocatalyst surface while filtering reactive oxygen species out of the biocatalyst environment, or that the high surface area crevices would unselectively adsorb and hinder necessary photosynthetic components with a negligible effect on reactive oxygen species.

In terms of redox polymers that could have the advantages of providing both polymeric matrix immobilization that may protect and stabilize biocatalysts as well as a method of mediating electron transport to the surface of the electrode, naphthoquinone and ferrocene linear modified polyethyleneimines (NQ-LPEI and Fc-LPEI) were chosen. Both NQ-LPEI and Fc-LPEI redox polymers had not yet been tested in photosynthetic systems, but were much cheaper than proven alternatives and had shown good results when applied with enzymes. NQ-LPEI was expected to be particularly effective at mediating thylakoid biocatalysts, as its naphthoquinone functional groups could successfully navigate through membranes and possibly imitate quinone electron acceptors already within the PSII photosystems abundantly located in the thylakoid membranes. Fc-LPEI was expected to be the more effective redox polymer at mediating PSI biocatalyst, as its redox potential is more favorable for pulling electrons away from the reducing protein and its iron-based functional groups might imitate the iron-sulfur

clusters already within the PSI electron transport chain.

These conditions were tested in comparison to blank biocatalyst electrodes and control catalase electrodes first by conducting amperometry on un-redox polymer modified thylakoid anodes to determine the optimal concentrations for the experimental reactive oxygen scavengers and then applying these optimal concentrations in conjunction with all the other variables to determine the photocurrent and lifetime data for all conditions. A comparison of all the photocurrent data collected for the various conditions can be seen in Figure 5-1, and a comparison of all the lifetime data collected can be viewed in Figure 5-2.

When initially determining optimal reactive oxygen scavenger concentrations using thylakoid amperometry testing, it was seen that activated carbon has a beneficial effect on lifetime at some concentrations with least uncertain lifetime preservation occurring at a concentration of 24 g/L, but that lifetime does not vary significantly with changing activated carbon concentration. At any concentration, the activated carbon electrodes showed photocurrents below those created by blank thylakoids with significantly lower photocurrents for source concentrations ranging between 6 g/L to 120 g/L, indicating that the poor selectivity of activated carbon to oxygen is allowing it to also adsorb organic molecules needed by the thylakoids to function properly and the opaque nature of the additive may be physically obstructing light from reaching the biocatalyst. Additionally, increasing concentrations of activated carbon correlate with increasing noise within electrode amperometry data, suggesting the great surface area of the activated carbon is providing too many competing pathways for photoelectron transferal. The lowest concentration of ascorbic acid tested (1mM) shows lifetimes

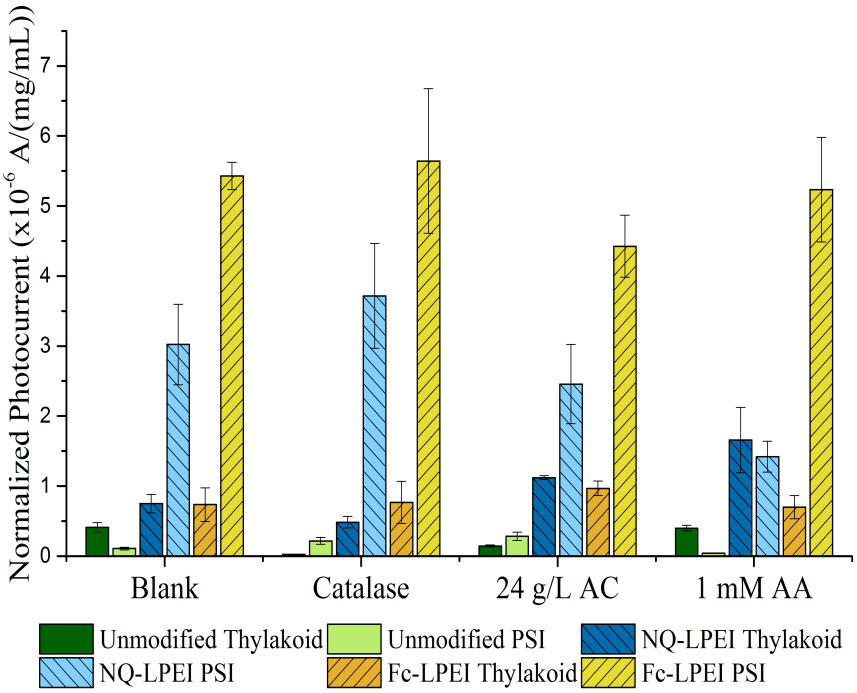

Figure 5-1. Comparison of the normalized photocurrents for the blank, catalase, and optimum experimental reactive oxygen scavenger concentration conditions for all biocatalyst and immobilization methods tested.

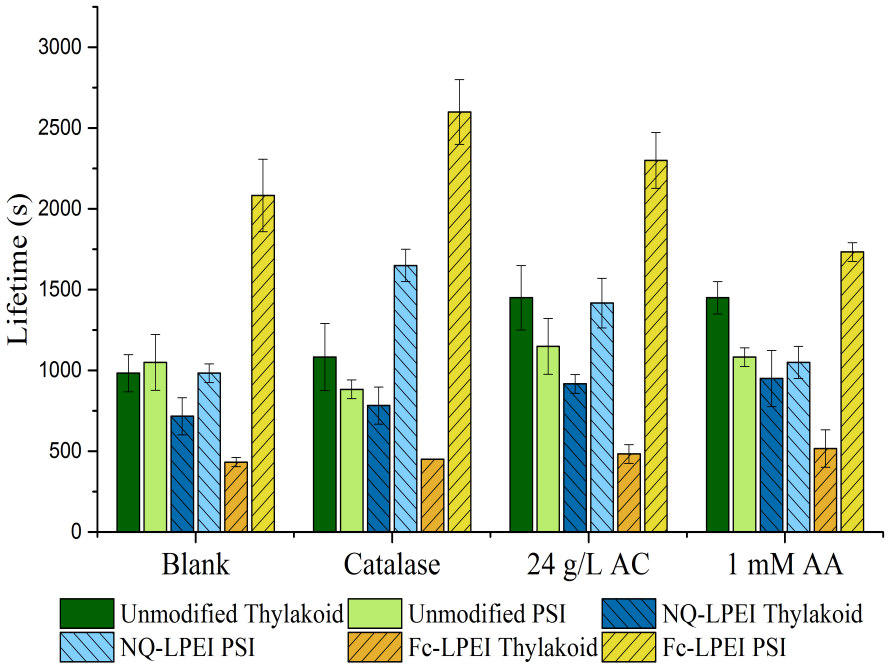

Figure 5-2. Comparison of the lifetimes for the blank, catalase, and optimum experimental reactive oxygen scavenger concentration conditions for all biocatalyst and immobilization methods tested.

comparable to the best lifetimes achieved by the activated carbon at 1450 ± 100s, or 148% of the unmodified blank thylakoid anode lifetimes, but with a greater significance in lifetime increase and significantly comparable photocurrents when compared with unmodified blank thylakoid anodes. However, higher concentrations of ascorbic acid tend to leach out of the electrodes and into the testing buffer. Both the 24 g/L activated carbon and the 1mM ascorbic acid experimental conditions offered the best performance increase over the blank thylakoid anodes and control catalase thylakoid anodes in terms of lifetime and were thus applied to the further test conditions involving redox polymer immobilizations and different biocatalyst conditions.

Both NQ-LPEI and Fc-LPEI modified thylakoid bio-anodes showed examples of increased electron transfer efficiencies with all conditions having significantly decreased biocatalyst stabilities when compared with their respective unmodified conditions. NQ-LPEI ascorbic acid conditions produced the highest photocurrents and estimated electron transfer efficiencies achieved out of any thylakoid biocatalyst anode condition, with a value 1103% of the unmodified blank thylakoid photocurrent and an efficiency of 1.437 ± 0.405%, although neither of these values were significantly different from the NQ-LPEI activated carbon thylakoid values. As expected due to the naphthoquinone functional groups' ability to transverse membranes and their similarity to the quinone electron acceptors in the PSII electron transport pathways, the NQ-LPEI redox polymer was best able to mediate electrons from thylakoids to the surface of the electrodes. Poor lifetimes of NQ-LPEI and Fc-LPEI modified anodes were believed to be due to the immobilization of thylakoid biocatalyst within the hydrogels. Immobilizing the thylakoids into unfavorably folded positions that more effectively trap photosynthetically

produced reactive oxygen species in close proximity to the thylakoid biocatalysts' essential photosynthetic components may significantly reduce electrode lifetime.

For photosystem I amperometry testing, it was seen that unmodified PSI bio-cathodes showed unexpectedly long lifetimes and no consistent evidence of significantly increased DET with the electrode surface when compared with unmodified thylakoid electrode results, contrary to hypothetical expectations. It is believed that the long lifetimes of the silica immobilized PSI despite its more fragile nature as isolated proteins is due to the fact that PSI creates no oxygen during its photosynthetic operations, unlike thylakoid membranes, and so does not trap any reactive oxygen species that could deteriorate the biocatalyst within the electrode system. In terms of lower than expected DET properties, it was later shown through comparison with a control polymer immobilization method using the non-redox reactive C8-LPEI that despite PSI's much thinner nature in comparison to thylakoid membranes, it was still not pressing as closely as possible to the electrode surface under unmodified, silica immobilized conditions.

Once the PSI biocatalyst was immobilized with redox polymer, it showed both significant increase in electron transfer efficiency and an increase in lifetime for most conditions when compared to unmodified blank PSI values. Fc-LPEI catalase PSI cathodes showed the highest average photocurrent of $5.642 \pm 1.033 \times 10^{-6}$ A/(mg/mL), estimated efficiency of $4.898 \pm 0.896\%$ (although neither of these values were significant from those of other Fc-LPEI conditions), and lifetime of 2600 ± 200 s (which was not significantly distinct from the Fc-LPEI activated carbon condition) recorded for any bio-electrode conditions tested for both biocatalysts. Both increased DET due to closer immobilization of PSI to the electrode surface by the polymer as well as successful MET

of electrons from the biocatalyst by the redox functional groups helped to provide these increases in electron transfer efficiency. Due to the similarity of the iron complex redox functional groups to the iron-sulfur complexes that are part of the PSI electron transport chain as well as its more favorable redox potential, the success of Fc-LPEI, in particular, at mediating PSI was expected over that of the NQ-LPEI redox polymer. The great increase in lifetime seen for both polymers is attributed not only to the lack of native reactive oxygen byproducts of photosynthesis, but also due to more beneficial immobilization of PSI by the redox polymers than was achieved for the thylakoids. No consistent significant improvement of stability was seen with the addition of reactive oxygen scavengers. Although Fc-LPEI catalase conditions showed the largest average lifetimes for any electrode tested, this lifetime was not significantly distinct from that of the Fc-LPEI activated carbon condition. Of the two experimental reactive oxygen scavenger conditions tested, both may have interfered with optimal electrode functionality, with activated carbon showing signs of preventing the best protein and polymer contact needed for mediation and stabilization and ascorbic acid results suggesting this additive may have been damaging both PSI protein and polymer structures.

Thus, in final summary, these experiments showed the importance in minimizing the concentration of reactive oxygen species when trying to improve stability of bio-solar photosynthetic electrodes and the fact that selecting appropriate redox polymer immobilization systems will greatly increase electron transfer efficiency through MET. For thylakoid bio-anodes, the greatest and least uncertain lifetime of 1450 ± 100 s was achieved by a combination of unmodified silica immobilization and 1mM ascorbic acid

reactive oxygen scavenger additive. This significantly represented 148% of the lifetime for the unmodified blank thylakoid bio-anode, the original starting and comparison point for all tested bio-electrodes in these experiments. The greatest thylakoid bio-anode electron transfer efficiency was achieved by the NQ-LPEI modified 1mM ascorbic acid condition, with a value of 1.656 ± 0.467 μA per mg/ml of chlorophyll. This had a value 1103% of the unmodified blank thylakoid photocurrent and an estimated electron transfer efficiency of 1.437 ± 0.405%, although neither photocurrent or efficiency values were significantly distinct from NQ-LPEI activated carbon thylakoid values. For PSI bio-cathodes, the Fc-LPEI immobilized catalase condition achieved both the greatest lifetime of 2600 ± 200 s (264% of the unmodified blank thylakoid condition) and the greatest photocurrent and estimated electron transfer efficiency of 5.642 ± 1.033 μA per mg/ml (1375% of the unmodified blank thylakoid condition) and 4.898 ± 0.896% observed for any electrode, either thylakoid or PSI biocatalyst. These high photocurrent and efficiency values were not significant from any of the other Fc-LPEI conditions, which all mediated PSI biocatalyst well, and the lifetime was not significantly distinct form that of Fc-LPEI activated carbon conditions. The results suggest that in terms of looking for a viable photosynthetic bio-solar cell electrode, more attention should possibly be focused on PSI than on thylakoid biocatalysts due to the increased stability PSI's lack of inherent reactive oxygen species production and favorable reaction to polymer immobilization can bring, as well as their greater electron transfer efficiency in conjunction with an appropriate mediating redox polymer like Fc-LPEI.

APPENDIX A

MATERIAL SOURCES AND BUFFER COMPOSITIONS

Material Sources

All chemicals and enzymes used in the production of buffers and solar cell electrodes obtained from Sigma Aldrich. NQ-LPEI, Fc-LPEI, and C8-LPEI polymers were synthesized by members of the Minteer lab group at the University of Utah. The spinach used to provide a source of biocatalysts was consistently obtained from the same Whole Foods Market store close to the University of Utah campus to minimize source variations in biocatalyst extractions.

Buffer Compositions

Thylakoid Extraction

Chloroplast Isolation Buffer (CIB) 5M for 1 L, pH = 7.8

1.65 M Sorbitol…….300 g

0.25 M 4-(2-hydroexyethyl)-1-piperazineethnesulfonic acid (HEPES)……..59.6 g

50 mM NaCl……..…2.9 g

13 mM Ethylenediaminetetraacetic acid (EDTA)…….3.7 g

53 mM $MgCl_2*6H_2O$…….……...5 g

Lysing Solution for 1 L, pH unmonitored

2 mM $MgCl_2*6H_2O$……...0.407 g

Thylakoid Resuspension Buffer (TRB) for 1 L, pH = 7.8

330 mM Sorbitol……....60.12 g

50 mM HEPES……...11.56 g

2 mM $MgCl_2*6H_2O$……...0.407 g

Photosystem I Extraction and Purification

Chloroplast Preparation Buffer for 1 L, pH = 7

50 mM Sodium Phosphate: Monosodium Phosphate……...……...……2.91 g

Disodium Phosphate…….....7.73 g

10 mM NaCl……..0.584 g

Solubilization Medium for 1 L, pH = 8.8

50 mM Trizma-HCl……7.88 g

3% Triton X-100…….30 mL

Starting Buffer for 1 L, pH = 8.8*

10 mM Trizma-HCl……...…1.576 g

0.2% Triton X-100……2 mL

20% Sucrose……200 g

*2 L of this buffer was made, then 500 mL were separated and modified to include a 200

mM concentration of NaCl (5.844 g for 500 mL of solution) to create both "no NaCl" and "200 mM NaCl" conditions of starting buffer mentioned in the procedures.

Hydroxyapatite Medium for 1 L, pH = 7.5

10 mM Trizma-HCl……...1.576 g

0.6 mM $CaCl_2$*$2H_2O$…….0.882 g

Equilibration Buffer for 1 L, pH = 7.5

10 mM Trizma-HCl……...1.576 g

0.3 mM $CaCl_2$*$2H_2O$…….0.441 g

0.05% Triton X-100……...0.5 mL

Elution Buffer for 1 L, pH = 8

50 mM Sodium Phosphate: Monosodium Phosphate……...……...……2.91 g

Disodium Phosphate…….....7.73 g

0.05% Triton X-100……...0.5 g

Testing Buffer

Citrate Buffer for 1 L, pH = 5.5*

0.1 M Sodium Citrate Dihydrate……...….29.41 g

0.1 M Citric Acid……...21.01 g

*Separate component solutions listed were mixed at a rough ratio of 2:1 until the desired pH was obtained.

APPENDIX B

ESTIMATED ELECTRON TRANSFER EFFICIENCY EXAMPLES

Unmodified Electrode Example Calculation

To begin estimating the efficiency of electrode electron transfer when compared to the most efficient (unextracted) biocatalyst condition, the rate of photosynthesis within spinach chloroplasts was found to be 119 μmol O_2/mg chlorophyll/h.[46] This was converted into a standard number of amperes per mg chlorophyll (A/mg Chl) using the Equation 1.

$$119 \times 10^{-6} \frac{\frac{mol\ O_2}{mg\ Chl}}{h} \times 4 \frac{mol\ electron}{mol\ O_2} \times 96485 \frac{C}{mol\ electron} \times \frac{1\ h}{3600\ s} = \mathbf{0.0128} \frac{\boldsymbol{A}}{\boldsymbol{mg\ Chl}} \quad \text{(Eq. 1)}$$

For electrodes unmodified by redox polymers, the normalized photocurrents (A/mg Chl/mL, or equivalently A*mL/mg Chl) were adjusted to the proper units of A/mg Chl by dividing them by the amount of mL biocatalyst used per electrode, or, in this case, 0.025 mL (equivalent to 25 μL). For example, the unmodified blank thylakoid normalized photocurrent was adjusted shown in Equation 2.

$$4.107 \times 10^{-7} \frac{A*mL}{mg\ Chl} \times \frac{1}{0.025\ mL} = 1.643 \times 10^{-5} \frac{A}{mg\ Chl} \quad \text{(Eq. 2)}$$

Percent efficiency was then calculated by dividing these adjusted photocurrents by the standard found for unextracted biocatalysts in Equation 1 and multiplying by 100 as seen in Equation 3.

$$1.643 \times 10^{-5} \frac{A}{mg\ Chl} \times \frac{1}{\frac{0.0128A}{mg\ Chl}} = 0.00128 \times 100 = \mathbf{0.128}\% \quad \text{(Eq. 3)}$$

Redox Polymer Modified Electrode Example Calculation

For electrodes modified by redox polymers, the process was the same, except that the normalized photocurrents were adjusted by a volume of 0.009 mL (9 μL) instead of 0.025 mL. For example, the calculations for the NQ-LPEI modified blank thylakoid normalized photocurrent are as seen in Equations 4 and 5.

$$7.503 \times 10^{-7} \frac{A*mL}{mg\ Chl} \times \frac{1}{0.009\ mL} = 8.337 \times 10^{-5} \frac{A}{mg\ Chl} \quad \text{(Eq. 4)}$$

$$8.337 \times 10^{-5} \frac{A}{mg\ Chl} \times \frac{1}{\frac{0.0128A}{mg\ Chl}} = 0.00651 \times 100 = \mathbf{0.651}\% \quad \text{(Eq. 5)}$$

REFERENCES

[1]Lewis, N. S.; Nocera, D. G. Powering the planet: Chemical challenges in solar energy utilization. *Proc. Natl. Acad. Sci. U.S.A.* **2006,** *103*, 15729-15735.

[2]Rasmussen, M.; Minteer, S. Photobioelectrochemistry: solar energy conversion and biofuel production with photosynthetic catalyst. *Jour. Electrochem. Soc.* **2014,** *161*, H647-H655.

[3]Aleksejeva, O. Photo-electrochemical communication between thylakoid membranes and electrodes for harnessing sunlight. M.S. Thesis, Lund University, Sweden, 2014.

[4]Calkins, J. O. Manipulating photosynthesis for energy conversion in an electrochemical cell. M.S. Thesis, Georgia Institute of Technology, Athens, GA, 2011.

[5]Ort, D. R.; Yocum, C. F. *Oxygenic Photosynthesis: The Light Reactions*, Springer Netherlands: Netherlands, 1996; Vol. 4, pp. 1-9.

[6]Ciesielski, P. N. Photosystem I – based systems for photoelectrochemical energy conversion. Ph.D. Dissertation, Vanderbilt University, Nashville, TN, August 2010.

[7]Rasmussen, M.; Wingersky, A.; Minteer, S. Comparative study of thylakoids from higher plants for solar energy conversion and herbicide detection. *Electrochim. Acta* **2014,** *140*, 304-308.

[8]Sjoholm, K. H.; Rasmussen, M.; Minteer, S. Bio-solar cells incorporating catalase for stabilization of thylakoid bioelectrodes during direct photoelectrocatalysis. *Jour. Electrochem. Soc.* **2012,** *1*, G7-G9.

[9]Rasmussen, M.; Minteer, S. Thylakoid direct photobioelectrocatalysis: utilizing stroma thylakoids to improve bio-solar cell performance. *Phys. Chem. Chem. Phys*. **2014,** *16*, 17327-17331.

[10]Rasmussen, M.; Wingersky, A.; Minteer, S. Improved performance of a thylakoid bio-solar cell by incorporation of carbon quantum dots. *Jour. Electrochem. Soc.* **2013,** *3*, H1-H3.

[11]Kerry, J.; Butler, P. *Smart Packaging Technologies for Fast Moving Consumer Goods*,

1st ed.; John Wiley and Sons Ltd.: West Sussex, England, 2008, pp.4-6.

[12]Eissa, A. A. *Structure and Function of Food Engineering*, 1st ed., Intech, 2012, pp. 21-33.

[13]Morissey, J.; Guerinot, M. L. Iron uptake and transport in plants: the good, the bad, and the ionome. *Chem. Rev.* **2009,** *109*, 4553-4567.

[14]Lenntech. Fenton's reaction. http://www.lenntech.com/fenton-reaction.htm (accessed Feb. 12, 2016).

[15]Grant, B. L. Sodium tolerance of plants- what are the effects of sodium in plants? https://www.gardeningknowhow.com/garden-how-to/soil-fertilizers/sodium-in-plants.htm (accessed Feb. 12, 2016).

[16]Guardian CSC. Oxygen scavengers. http://www.guardiancsc.com/pdf/5_02_scavengers.pdf (accessed Feb. 12, 2016).

[17]Chelikani, P.; Fita, I.; Loewen, P. C. Diversity in structures and properties among catalases. *Cell. Mol. Life Sci.* **2004,** *61*, 192-208.

[18]Kim, S.; Lee, I.; Hong, S. The effect of oxygen scavenging system on the pH of buffered sample solutions: in the context of single-molecule flourescence measurements. *Bull. Korean Chem. Soc.* **2012,** *33*, 958-962.

[19]Enzyme-based oxygen scavengers based on glucose oxidase/catalase. http://www.foodtech-portal.eu/index.php?title=Enzyme-based_oxygen_scavengers_based_on_glucose_oxidase/catalase (accessed Feb. 12, 2016).

[20]Leskovac, V.; Trivic, S.; Wohlfahrt, G.; Kandrac, J.; Pericin, D. Glucose oxidase from *Aspergillus niger*: the mechanism of action with molecular oxygen, quinones, and one-electron acceptors. *Int. J. Biochem. Cell Biol*. **2005,** *37*, 731-750.

[21]Bankar, S. B.; Bule, M. V.; Singhal, R. S.; Ananthanarayan, L. Glucose oxidase – an overview. *Biotech. Advances* **2009,** *27*, 489-501.

[22]Goswami, P.; Chinnadayyala, S. S. R.; Chakraborty, M.; Kumar, A. K.; Kakoti, A. An overview on alcohol oxidases and their potential applications. *Appl. Microbiol. Biotechnol.* **2013,** 1-17.

[23]Potters, G.; De Gara, L.; Asard, H.; Horemans, N. Ascorbate and glutathione: guardians of the cell cycle, partners in crime? *Plant Physiol. Biochem*. **2002,** *40*, 537-548.

[24]Forti, G.; Elli, G. The function of ascorbic acid in photosynthetic phosphorylation. *Plant Physiol.* **1995,** *109*, 1207-1211.

[25]Dave, R.; Shah, N. P. Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures. *Int. Dairy Journal* **1997,** *7*, 435-443.

[26]General Carbon Corporation. Cleaning the world with activated carbon. http://generalcarbon.com/facts-about-activated-carbon/ (accessed Feb. 12, 2016).

[27]Chemistry Explained. Carbon. http://www.chemistryexplained.com/elements/A-C/Carbon.html (accessed Feb. 12, 2016).

[28]Which type of activated carbon do you recommend for gas adsorption applications – granular or powdered? https://www.researchgate.net/post/Which_type_of_activated_carbon_do_you_recommend_for_gas_adsorption_applications-granular_or_powdered (accessed Feb. 12, 2016).

[29]Laberge, D.; Rouillon, R.; Carpentier, R. Comparative study of thylakoid membranes sensitivity for herbicide detection after physical or chemical stabilization. *Enzyme Microb. Technol.* **2000,** *26*, 332-336.

[30]Lemieux, S.; Carpentier, R. Properties of immobilized thylakoid membranes in a photosynthetic photoelectrochemical cell. *Photochem. Photobiol*. **1988,** *48*, 115-121.

[31]Carpentier, R.; Lemieux, S., Mimeault, M.; Photocurrent generation by thylakoid membranes immobilized in an albumin-glutaraldehyde cross-linked matrix. *Biotechn. Lett.* **1988,** *10*, 133-136.

[32]Carpentier, R.; Leblanc, R. M.; Mimeault, M.; Photoinhibition and chlorophyll photobleaching in immobilized thylakoid membranes. *Enzyme Microb. Technol*. **1987,** *9*, 489-492.

[33]Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley & Sons, Inc.: New York, 2001; pp. 580-631.

[34]Lam, K. B.; Irwin, E. F.; Healy, K. E.; Lin, L. Bioelectrocatalytic self-assembled thylakoids for micro-power and sensing applications. *Sens. Act. B* **2006,** *117*, 480-487.

[35]Trammell, S. A.; Griva, I.; Spano, A.; Tsoi, S.; Tender, L. M.; Schnur, J.; Lebedev, N. Effects of distance and driving force on photoinduced electron transfer between photosynthetic reaction centers and gold electrodes. *J. Phys. Chem.* **2007,** *111*, 17122-17130.

[36]Terasaki, N.; Iwai, M.; Yamamoto, N.; Hiraga, T.; Yamada, S.; Inoue, Y. Photocurrent generation properties of histag-photosystem II immobilized on nanostructured gold electrode. *Thin Sol. Films* **2008**, *516*, 2553-2557.

[37]Hamidi, H.; Hasan, K.; Emek, S. C.; Dilgin, Y.; Akerlund, H.; Albertsson, P.; Leech,

D.; Gorton, L. Photocurrent generation from thylakoid membranes on osmium-redox-polymer-modified Electrodes. *Chem. Sus. Chem.* **2015,** *8*, 990-993.

[38]Hickey, D. P. Ferrocene-modified linear poly(ethylenimine) bioelectrode materials for use in glucose/O_2 biofuel cells. Ph.D. Dissertation, University of Oklahoma, Norman, OK, 2014.

[39]Milton, R. D.; Hickey, D. P.; Abdellaoui, S.; Lim, K.; Wu, F.; Tan, B.; Minteer, S. D. Rational design of quinones for high power density biofuel cells. *Chem. Sci.* **2015,** *6*, 4867-4875.

[40]Abdellaoui, S.; Milton, R. D.; Quah, T.; Minteer, S. D. NAD-dependent dehydrogenase bioelectrocatalysis: the ability of a naphthoquinone redox polymer to regenerate NAD. *Chem. Commun.* **2016,** *52*, 1147-1150.

[41]Saleem, M.; Yu, H.; Wang, L.; Abdin, Z.; Khalid, H.; Akram, M.; Abbasi, N. M.; Huang, J. Review on synthesis of ferrocene-based redox polymers and derivatives and their application in glucose sensing. *Anal. Chim. Acta* **2015,** *876*, 9-25.

[42]Peitschnig, R. Polymers with pendant ferrocenes. *Chem. Soc. Rev.* **2016,** *45*, 5216-5231.

[43]Drazer, G.; Chertcoff, R.; Bruno, L.; Rosen, M. Concentration dependence of diffusion-adsorption rate in activated carbon. *Chem. Eng. Sci.* **1999,** *54*, 4285-4291.

[44]Racyte, J.; Langenhoff, A. A. M.; Ribeiro, A. R. M. M. R.; Paulitsh-Fuchs, A. H.; Bruning, H.; Rijnaarts, H. H. M. Effect of granular activated carbon concentration on the content of organic matter and salt, influencing *E. coli* activity and survival in fluidized bed disinfection reactor. *Biotechnol Bioeng*. **2014,** *111*, 2009-2018.

[45]Harris, D. C. *Quantitative Chemical Analysis*, 7th ed.; W. H. Freeman and Company: New York, 2007; pp. 53-77.

[46]Robinson, S. P.; Downton, W. J. S.; Millhouse, J. A.; Photosynthesis and ion content of leaves and isolated chloroplasts of salt-stressed spinach. *Plant Physiol.* **1983,** *73*, 238-242.